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THE PHOSPHORUS-CONTAINING SUBSTANCES OF THE CELL.

OUR knowledge of the form and position in which the phosphorus is held in the animal cell has of recent years become considerably advanced by the researches of Miescher, Kossel and their co-workers, and has been greatly stimulated by the discovery of Altmann, that from the chief and most abundant of the phosphorus proteids the phosphorus can be split off in the form of a complex acid which he has called *nucleic acid*. The constitution of nucleic acid has now been made the subject of a good deal of work which has led to all the more promising results, because though it is a very complex body, the size of its molecule is small as compared with those of the highly complex proteids by which it is accompanied. In this short paper we will first give an account of nucleic acid itself so far as it is at present known and then discuss the compounds in which form it is present in the cell.

NUCLEIC ACID.

Nucleic acid was first prepared and described by Altmann.[1] He obtained it best from yeast by treating the cells for about five minutes with a large volume of 3 per cent. NaOH. The alkali was then nearly neutralised with hydrochloric acid and an access of acetic acid added. Most of the proteid was thus precipitated, whilst the

[1] Altmann: *Arch. für (Anat. u.) Phys.*, 1889, p. 524.

nucleic acid remained in solution and was obtained by adding hydrochloric acid up to 0·3 per cent. and then throwing it into an equal volume of alcohol containing the same amount of hydrochloric acid when the acid was precipitated. He also obtained similar bodies from thymus and egg-yolk. He showed that they all contained phosphorus varying in amount from 7·9 per cent. to 9·5 per cent. and that sulphur was absent if the acid had been completely separated from all proteid.

Nucleic acid as thus prepared was found to be a white amorphous powder giving a strong acid reaction. It dissolves fairly readily in water and very readily if a little ammonia or other alkali be added. From this solution it is not precipitated by an excess of acetic acid but is by weak hydrochloric, especially in the presence of alcohol. It is quite insoluble in alcohol and ether.

As early as 1874 Miescher[1] described the preparation and properties of a substance rich in phosphorus and of marked acid properties which he had obtained from the nuclei of salmon spermatozoa. This substance Miescher regarded as a nuclein, but as was pointed out by Kossel he was in reality dealing with nucleic acid. In his later work, collected and published by Schmiedeberg[2] after the author's death, Miescher recognises this and gives his further work upon the chemistry of the acid. Nucleic acid is particularly characterised by its instability and Miescher points out two great dangers which have to be avoided if we wish to obtain a preparation of unaltered nucleic acid. In the process of purification it is extremely liable to decompose, with the result that it loses a considerable part of its phosphorus. In the second place it is most easily split up in another manner in which it loses a considerable part of its nitrogen which is given off in the form of the so-called nuclein bases or alloxuric bodies, xanthin, hypoxanthin, etc. This latter decomposition occurs especially during that part of the preparation when it is necessary to employ dilute mineral acids,

[1] Miescher: *Verhandl. d. naturf. Ges. in Basel*, Bd. 6, S. 138, 1874, and *Ber. d. d. chem. Ges.*, Bd. 7, S. 1714, 1874.

[2] Miescher: *Arch. f. exp. Path. u. Pharm.*, Bd. 37, S. 100, 1896.

and though Miescher showed that it was easy to prevent the loss of any of the phosphorus, it was much more difficult to prevent the loss of any of the nitrogen. To avoid the latter source of error he found that it was necessary to keep the temperature of all solutions down to 0° C., the whole time of the preparation, and particularly during the treatment with dilute acid, the time of which it is further necessary to reduce to the lowest possible limit. His method is to take the ripe spermatozoa and rub them up in water. The emulsion is then strained through a cloth, precipitated by adding a few drops of acetic acid, and filtered. The precipitate is washed thoroughly with alcohol and ether to remove fats, lecithin, etc., and then thoroughly shaken with 0·5 per cent. hydrochloric acid and this extraction repeated four or five times. It is in the later extractions that the danger of splitting off some of the nuclein bases is especially incurred. The residue is next dissolved in ¼ per cent. soda, filtered, and the filtrate acidified with hydrochloric acid when the nucleic acid is partially precipitated, but only completely on the addition of two volumes of alcohol. During the whole of these processes the preparation and all reagents added are cooled down to about 0° C. A general and striking reaction given by nucleic acid is that in acid solutions it will precipitate proteids, producing bodies which, as Altmann showed, very closely resemble if they are not identical with the nucleins obtainable from most tissues.

The importance of the part that nucleic acid plays in the animal economy, either when united with proteid molecules to form compound proteids or in a free state, stands forth clearly when we study the nature of the decomposition products which have been obtained from it, or, on the other hand, by a study of the positions from which it has been obtained. Thus its distribution is found to be chiefly, if not entirely, in the nuclei of cells. The chromatin of the nuclei appears to be the chief source of the acid, as is seen from Miescher's and other experiments in which the nuclei have been isolated in quantity and found to consist almost entirely of nucleic acid combined with a base, protamine. This conclusion is further strengthened by a study of the

reactions to staining agents. Thus on staining different substances with the Ehrlich-Biondi triple stain Kossel[1] found that proteids select the acid stain, *i.e.*, the fuchsine, that nucleic acid on the other hand takes the basic stain, the methyl-green. On staining resting nuclei with the triple stain they are tinted of a violet colour, and therefore Kossel argues contain the nucleic acid combined with proteid; on the other hand, in cells that are subdividing, the nuclei stain a green colour, showing that a large part of the nucleic acid is probably in a free state. The same reaction to stains is shown by the nuclei of freshly formed spermatozoa.

It is, however, with the constitution of nucleic acid that we are here chiefly interested, and our knowledge so far depends upon the decomposition products that have been obtained from it by various methods. The whole of the phosphorus may be separated as phosphoric acid by boiling it for a short time with dilute sulphuric acid. According to Liebermann[2] the phosphorus exists in the nucleic acid molecule in the form of metaphosphoric acid, for he obtains it as such by heating for a short time with dilute nitric acid. That it is not all held in the same way in the molecule is shown by the fact that one part of it can be readily split off from the molecule by simply heating it for a time in water. The major part, however, under such treatment remains unaffected.

Since Kossel first showed that the nuclein prepared from yeast yielded xanthin and hypoxanthin, and at a later time that the nucleic acid molecule was the source of these bases, many nucleic acids have been examined for these alloxuric bodies, so that now the identification of a nucleic acid depends upon proving the existence of one or more of these bases as a result of decomposition of the moleule. The chief members of this group of alloxuric bases are as follows:—

1. *Xanthin*, $C_5H_4N_4O_2$, which has been obtained from

[1] Kossel: *Deutsche. med. Woch*, 1894, No. 7.

[2] Liebermann: *Pflüger's Arch.*, Bd. 47, S. 155, 1890; and *Ber. d. d. Chem. Ges.*, Bd. 21, S. 598, 1888.

very many organs, *e.g.*, from the nuclein of yeast (Kossel [1]), from the nucleic acid prepared from ox-spermatozoa, or again from that of carp-spermatozoa. It has also been obtained from the spleen, the pancreas, the thymus, etc.

2. *Guanin*, $C_5H_5N_5O$, has been prepared from the nuclein of yeast (Kossel), and from the nucleic acid prepared from thymus (Kossel), and from that of salmon-spermatozoa (Miescher). It has also been obtained from numerous cellular organs, *e.g.*, liver, spleen, pancreas, etc. It is closely related to xanthin, into which it may be converted by the action of nitrous acid when an imido-group (=NH) of the guanin is split off and an O atom inserted in its place.

3. *Hypoxanthin*, $C_5H_4N_4O$, has been found in the same position as xanthin, and in especial quantity in salmon sperm (Miescher). It has been prepared from the nuclein of leucocytes (Kossel [2]) and of yeast (Kossel [3]).

4. *Adenin*, $C_5H_5N_5$, was first discovered by Kossel, who obtained it from the pancreas. He further obtained it from thymus-nucleic acid [4] and from the nuclein of yeast. It stands in the same relationship to hypoxanthin that guanin does to xanthin.

$$C_5H_5N_5 + HNO_2 = C_5H_4N_4O + N_2 + H_2O$$

and $$C_5H_5N_5O + HNO_2 = C_5H_4N_4O_2 + N_2 + H_2O.$$

The finding of these bases in this position is particularly of interest in conjunction with their close relationship to uric acid ($C_5H_4N_4O_3$), which only differs from xanthin in possessing one more oxygen atom. Their molecular structure too is similar, and just as with uric acid they are found to split up into alloxan, parabanic acid, etc., under the influence of oxidising agents. With regard to the part they may play in the formation of uric acid in the body we will return later.

Cytosin.—Associated with these bases though not showing exactly the same properties is another base,

[1] Kossel: *Ztschr. f. physiol. Chem.*, Bd. 4, S. 290, 1880.

[2] Kossel: *Ibid.*, Bd. 5, S. 152, 1881.

[3] Kossel: *Ibid.*, Bd. 4, S. 290, 1880.

[4] Kossel: *Ber. d. d. chem. Ges.*, Bd. 26, S. 2753, 1893.

cytosin, which Kossel[1] has obtained from thymus-nucleic acid in conjunction with adenin and guanin when the acid is boiled for twenty minutes in water. This base has a formula $C_{21}H_{30}N_{16}O_4$ 5 aq, and is precipitated by phosphotungstic acid, and forms crystalline salts with sulphuric and hydrochloric acids, and with platinum chloride or gold chloride. It is precipitated in the form of a silver compound by an ammoniacal solution of silver oxide. It was obtained to the amount of 2 per cent. of the nucleic acid from which it was derived.

We thus see that with regard to the nuclein bases they yield, the nucleic acids prepared from different organs have different constitutions, and Kossel suggests that for each base there is a corresponding nucleic acid, there being at least four of these. For these acids he suggests the names xanthylic, adenylic, etc. We cannot, however, as yet consider this as proven, the alternative that a nucleic acid may have one or more of different basic molecules within it being quite as probable, and, moreover, it explains the facts just as well as the other supposition. It is probable too that far greater differences lie in the other parts of the nucleic acid molecule than the variation in the alloxuric base with which it is combined. A further point of some importance is that though in these nucleic acids we are dealing with a compound of a body of distinct acid properties with a base, yet it is not of the nature of a salt. Kossel and Neumann[2] have shown that if thymus-nucleic acid be boiled in water for about five minutes the acid is split up into the nuclein bases, in this instance adenin together with smaller quantities of guanin and cytosin, and a phosphorous rich acid which they term *thyminic acid.* Thyminic acid contains no further nuclein base, but if an alloxuric base is added to its solution a thyminate of the base is found. This, however, differs from the original nucleic acid, for if the two substances are treated with barium hydrate and the resulting solutions precipitated by throwing

[1] Kossel: *Ber. d. d. chem. Ges.*, Bd. 27, S. 2215, 1894, and *Arch. f. (Anat. u.) Physiol.*, 1894, S. 551.

[2] Kossel and Neumann: *Zeitschr. physiol. Chem.*, Bd. 22, S. 81, 1896.

them into alcohol the filtrate in the case of the thyminate is found to contain the nuclein base, whereas in the case of the nucleic acid no base is split off.

Turning next to another group of bodies, it has been found that some of the nucleic acids yield *carbohydrates* among their decomposition products. Thus, working on the nucleic acid of yeast, Kossel[1] obtained evidence of a carbohydrate in that molecule, and in a later paper[2] describes the preparation of an osazone melting between 204° and 205° C. and of a second melting at 105° C. The first he shows to be due to a glucose which does not ferment with yeast, but which reduces Fehling's solution. The second osazone is probably due to a pentose as shown by the production of furfurol from it. It is to be noted that the carbohydrate molecule belongs to the nucleic acid part of the nuclein not to the proteid portion. He was not able to obtain any carbohydrate from the nucleic acid prepared from salmon spermatozoa. Hammarsten[3] describes the preparation of a substance from ox-pancreas, very closely related to the nucleins, from which he was able to obtain a body reducing Fehling's solution by boiling the preparation with weak sulphuric acid. From the decomposition products he prepared an osazone melting at 158° to 160° C., easily soluble in alcohol or ether and which could be crystallised from its alcoholic solution by adding warm water and then allowing it to cool. The sugar did not ferment with yeast and its osazone did not correspond with that of any known sugar. It is probably a pentose. Another nucleic acid which has given evidence of the presence of a carbohydrate is that prepared from the thymus. On heating this for two hours in a Papin's digester at 150° C. with 20 per cent. sulphuric acid, Kossel and Neuman[4] found levulinic acid among the bodies into which it had been decomposed; and

[1] Kossel: *Arch. f.* (*Anat. u.*) *Physiol.*, 1891, S. 181.

[2] Kossel: *Ibid.*, 1893, S. 157.

[3] Hammarsten: *Zeitschr. Physiol. Chem.*, Bd. 19, S. 19, 1894.

[4] Kossel u. Neumann: *Ber. d. d. chem. Gesell.*, Bd. 27, S. 2215, 1894; and Kossel: *Arch. f.* (*Anat. u.*) *Physiol.*, 1894, S. 536.

from the presence of this acid they infer the existence of a carbohydrate group in the original substance.

By obtaining nuclein bases we are able to separate some of the nitrogen of nucleic acid, but a large part still remains to be accounted for. In investigating thymus nucleic acid Kossel and Neumann[1] have shown that by dropping it into boiling water in about ten minutes it is split up into its nuclein bases and a new acid, *thyminic acid.* To isolate the latter the watery solution is cooled and an excess of barium hydrate added. It is then allowed to stand for twenty-four hours when a precipitate slowly forms, which consist of guanin mixed with some barium carbonate. This is filtered off and the filtrate added to twice its volume of alcohol when a white precipitate of barium thyminate is thrown down and the adenin and cytosin remain in solution. Barium thyminate is a very hygroscopic salt, easily soluble in water, to which they assign the formula $C_{16}H_{23}N_3P_2O_{12}Ba$. Thyminic acid, as prepared from this salt, differs in several important respects from the nucleic acid from which it has been obtained. In the first place it does not yield any nuclein bases on being boiled with dilute sulphuric acid. It is very soluble in water, whereas nucleic acid is only feebly soluble; it is not precipitated from its watery solution by dilute mineral acids whereas nucleic acid is, both give precipitates when added to an acetic acid solution of a proteid or propeptone, but the precipitate given by nucleic acid is only soluble with difficulty in dilute mineral acids, whilst that of thyminic acid is easily dissolved by hydrochloric acid or by many neutral salts. They consider that this thyminic acid is a paranucleic acid and is probably identical with the acids obtained from some of the paranucleins. It is important to note that in this acid the ratio of the nitrogen to the phosphorus atoms is 3 : 2, whereas in nucleic acid it is 6 : 2, so that by decomposing nucleic acid in this way one half of the nitrogen is split off in the form of nuclein bases.

Some of the nitrogen of the thymus nucleic acid, or thyminic acid, has also been obtained in the form of a

[1] Kossel u. Neumann: *Ber. d. d. chem. Gesell.*, Bd. 26, S. 2753, 1893; and *Zeitschr. f. physiol. Chem.*, Bd. 22, S. 74, 1896.

crystalline substance which has been named *thymin*. On boiling the nucleic acid with 30 per cent. sulphuric acid or heating it under pressure in water to 170° C. Kossel and Neumann[1] were able to isolate this substance from the resulting solution. It is only slightly soluble in cold but easily soluble in hot water. It is also dissolved by hot alcohol. It has a bitter taste, and on careful heating it sublimes. It possesses neither acid nor basic properties, is not precipitated by phospho-tungstic acid, but is by mercuric chloride, or an ammoniacal solution of silver oxide. Its formula is $C_5H_6N_2O_2$, and it is therefore isomeric with methyl-uracil. They also prepared it from the nucleic acids obtained from yeast and from the spleen. In working upon the nucleic acid obtained from salmon spermatozoa Miescher[2] also obtained a non-basic body of the formula $C_5H_6N_2O_2$, to which Schmiedeberg gives the name nucleosin. This body has also been prepared from the same source by Kossel,[3] who has shown that it is identical with the thymin previously described by him.

Another body which has been obtained as the result of partial decomposition of the nucleic acid of yeast is a phosphorus-containing acid which Kossel[4] terms *plasmic acid*. This was obtained as the result of the action of dilute alkalies on nuclein at ordinary temperatures. The proteid part of the molecule is gradually split off from the nuclein, and bodies richer in phosphorus are thus obtained, of which the most complex is plasmic acid. It differs from nucleic acid by its solubilities, being readily dissolved by water or dilute hydrochloric acid and can thus be easily separated from nucleic acid. Like nucleic acid, it precipitates proteids in acid solution, and thus closely resembles thyminic acid, from which, however, it differs, in that nuclein bases are found among its decomposition products after it has been

[1] Kossel u. Neumann: *Ber. d. d. chem. Gesell.*, Bd. 26, S. 2753, 1893; and Bd. 27, S. 2215, 1894. See also *Arch. f.* (*Anat. u.*) *Physiol.*, 1894, pp. 194, 536 and 551.

[2] Miescher: *Arch. f. exp. Path. u. Pharm.*, Bd. 37, S. 125, 1896.

[3] Kossel: *Zeitschr. f. physiol. Chem.*, Bd. 22, S. 188, 1896.

[4] Kossel: *Arch. f.* (*Anat. u.*) *Physiol.*, 1893, p. 157.

boiled with dilute sulphuric acid. It shows a further difference from the nucleic acid from which it is obtained by yielding no carbohydrate molecule. It has not at present been prepared from nucleic acids derived from other sources. Its formula is given as $C_{15}H_{28}N_6P_6O_{30}$, and thus shows a relatively higher amount of phosphorus compared to nitrogen than either nucleic or thyminic acids.

We see then that nucleic acid is a very complex body which is moreover characterised by the ease with which it can be broken down into simpler and more stable molecules. Hence its preparation for purposes of analysis requires considerable care, and the formulæ which have been given for it can only at present be regarded as approximate. Miescher's formula for "salmonucleic acid" as recorded by Schmiedeberg[1] is $C_{40}H_{54}N_{14}P_4O_{27}$ and for a sample of yeast nucleic acid prepared by Altmann, Miescher[2] obtained the formula $C_{40}H_{54}(OH)_5N_{14}P_4O_{27}$ which only differs from that of salmonucleic acid by 5 (− OH). The formulæ given by Kossel are somewhat different. Thus for nucleic acid from yeast he gives[3] either $C_{17}H_{26}N_6P_2O_{14}$ or $C_{25}H_{36}N_9P_3O_{20}$, and for that from the thymus[4] $C_{30}H_{52}N_9P_3O_{17}$. Recently Mathews[5] working under Kossel has prepared a nucleic acid from the spermatozoa of an Echinoderm, *Arbacia*, and finds that the analyses of this agree completely with the later formula given by Miescher for that from salmon spermatozoa.

THE COMPOUNDS OF NUCLEIC ACID FOUND IN DIFFERENT CELLS.

Having considered the structure of nucleic acid we may next examine the different combinations in which it is found in different cells. In different positions they are found combined with proteids, or with bases belonging to the group

[1] Miescher: *Arch. f. exp. Path. u. Pharm.*, Bd. 37, S. 121, 1896.

[2] *Loc. cit.*, S. 122.

[3] Kossel: *Arch. f. (Anat. u.) Physiol.*, 1891, S. 181.

[4] Kossel: *Ibid.*, 1893, S. 157.

[5] Mathews: *Zeitschr. f. chem. Physiol.*, Bd. 23, S. 399, 1897.

of protamines or they exist in the free state. Their simplest compounds with proteids form the group of the *nucleins*, a name first applied to the substance isolated by Miescher from the nuclei of pus cells, being the residue left on submitting the cells to gastric digestion. The nucleins are found to be present in nearly all cells, though not in a free state, but combined with a further amount of proteid to form the group of bodies termed the *nucleo-proteids*. The nucleo-proteids react as weak acids whose neutral alkaline salts are soluble in water and coagulate on heating. If the solution or the heat coagulum be digested with artificial gastric juice most of the proteid is split off and dissolved leaving an insoluble residue, the nuclein. From the nuclein, as Altmann showed, we can, by the use of alkali, split off a further amount of proteid and thus obtain the nucleic acid. The nucleo-proteids are at present regarded as the most characteristic proteids obtainable from nuclei.

Closely related to the nucleins is another group of bodies containing phosphorus and showing many other points in common but differing in that they do not contain any nuclein base. To this group of bodies Kossel gives the name of *paranuclein* but for them Hammarsten[1] suggests as a more satisfactory term the name *pseudo-nuclein*. As the nucleins are found in the cell or nucleus combined with proteids as nucleo-proteids, so the paranucleins are also found combined with proteids. To these proteids Hammarsten suggests that the name of *nucleo-albumins* should be limited. Examples of the nucleo-albumins are casein and ovo-vitellin.

On artificial gastric digestion they leave an insoluble residue of paranuclein, but if the digestive fluid be strong the paranuclein gradually dissolves. The amount of phosphorus found in solution varies with the strength of the digestive fluid and with a smaller amount of the nucleo-albumin submitted to digestion. As a nuclein is split up into nucleic acid and a proteid so a paranuclein breaks down into a proteid and paranucleic acid, but this latter has not yet been much examined. Kossel and Neumann consider

[1] Hammarsten: *Ztschr. f. physiol. Chem.*, Bd. 19, S. 19, 1894.

the thyminic acid which they have isolated by removing the nuclein bases from thymus nucleic acid to be a paranucleic acid, but this cannot be considered proved until we know more about the solubilities and decomposition products of paranucleic acids prepared directly from casein and similar bodies.

The nucleo-albumins in contradistinction to the nucleo-proteids are apparently contained in the cell protoplasm and behave as strong acids, insoluble in water but readily dissolving in weak alkali. A neutral solution of their alkaline compound does not coagulate on heating and they apparently always contain iron and less sulphur than ordinary proteids.

In a recent paper Milroy[1] has examined the bodies obtained by precipitating acid solutions of proteids with nucleic and thyminic acids. The former Altmann considered as identical with the nucleins, and Milroy shows that these artificial nucleins and paranucleins give many of the reactions of the true nucleins and paranucleins, *e.g.*, their solubilities, percentage of phosphorus and behaviour to digestive ferments are the same; this resemblance however, is not sufficient to establish complete identity.

PROTAMINE.

Another compound of nucleic acid is with the *protamine* bases. So far these have only been found in the nuclei of the spermatozoa of fishes. The protamines are nitrogenous bases, only found at present in this position, and have recently been considerably studied in this connection.

The nucleic acid in the nuclei of the salmon spermatozoa appears to be present combined with a base which Miescher[2] termed protamine, and to which he assigned the formula $C_9H_{21}N_5O_3$. It forms about one-fourth of the total weight of the dried spermatozoa heads, and was isolated from them by repeated extraction with dilute hydrochloric acid (about

[1] Milroy: *Ztschr. f. physiol. Chem.*, Bd. 22, S. 307, 1896.

[2] Miescher: *Verhandl. d. naturf. Gesell. in Basel*, Bd. 6, S. 138, 1874; and *Ber. d. d. chem. Ges.*, Bd. 7, S. 396, 1874.

5 p.m.). The extract was then neutralised and platinum chloride added, when an amorphous precipitate of protamine platinum chloride is obtained. The free base possesses marked basic properties; is soluble in water but insoluble in alcohol or ether. It is precipitated by phospho-tungstic acid, potassio-mercuric iodide, mercuric chloride and gold chloride. The examination of this body was continued by Piccard,[1] who found that Miescher's preparation contained small quantities of guanin and hypoxanthin, and by fresh analysis of the purified platinum salt obtained the formula $C_{16}H_{32}N_9O_4$.

Twenty years later Kossel[2] described an important reaction of protamine, for he found that in an ammoniacal solution of a proteid or albumose protamine gave a precipitate which showed all the reactions of *histon*, a proteid which he had previously obtained[3] from the red corpuscles of birds' blood.

In Miescher's later paper further analyses of protamine are given from which the formula $C_{16}H_{28}N_9O_2$ is obtained. To investigate the constitution of protamine he heated the hydrochlorate up to 170° C. in a sealed tube with 15 per cent. hydrochloric acid, and among the decomposition products was able to isolate the base *Arginine* $C_6H_{14}N_4O_2$, which, originally discovered by Schultze and Steiger[4] in vegetable tissues, has since been isolated by Hedin[5] from the decomposition products of many proteids of animal tissues. The great interest of this base lies in the fact that if its silver compound be boiled with baryta water for twenty minutes it yields urea. Hedin also shows that the base lysatine obtained by Drechsel from proteids, which similarly yields urea, is in reality a mixture of lysine with arginine.

[1] Piccard: *Ber. d. d. chem. Ges.*, Bd. 7, S. 1714, 1874.

[2] Kossel: *Deutsche med. Wochenschr.*, 1894, No. 7.

[3] Kossel: *Zeitschr. f. physiol. Chem.*, Bd. 8, S. 511, 1884.

[4] Schultze and Steiger: *Ztschr. f. physiol. Chem.*, Bd. 9, S. 43; and *Ber. d. d. chem. Ges.*, Bd. 24, S. 2707; and Bd. 29, S. 352, 1896.

[5] Hedin.: *Ztschr. f. physiol. Chem.*, Bd. 20, S. 186, 1895; Bd. 21 S. 155, 1896; and Bd. 22, S. 191, 1897.

The method Kossel[1] recommends for the preparation of protamine is to thoroughly extract the dried spermatozoa heads, which have been previously freed from fat, etc., by extraction with alcohol and ether, with 1 per cent. sulphuric acid. This is then filtered and added to three times its volume of alcohol, when a precipitate of protamine sulphate is formed and can be readily purified. From analysis of the pure salt he arrives at the formula $C_{16}H_{31}N_9O_3$ for the base. He criticises Miescher's formula, showing that it cannot be correct because it does not satisfy the necessary condition that the sum of the valencies should be an even number, and points out that if another H atom be added to Miescher's formula, then the two only differ by one molecule of water. For the protamine obtained from salmon sperm he suggests the name *salmine*, and for a similar protamine obtained from the spermatozoa of the sturgeon, the name *sturine*. If sturine be decomposed by boiling it for eight hours with 30 per cent. sulphuric acid and then the sulphuric acid be removed, an alkaline fluid is left which gives a precipitate with mercuric chloride, leaving further bases still in solution. The mercuric precipitate is a compound of a new base which Kossel terms *histidine* having a formula $C_6H_9N_3O_2$, which is readily soluble in water, but crystallises out when an equal volume of alcohol is added to its solution. It is quite insoluble in ether. Histidine has also been found by Hedin[2] among the decomposition products from casein, egg-white and vitellin. From the filtrate from the histidine precipitate Kossel also prepared arginine, thus confirming and extending the work of Miescher upon salmine. Kossel further examined the decomposition products of sturine for *amido-acids*, but found that they were only present in quite minimal amounts, so that practically the whole of the nitrogen of the protamines is present in the form of bases. Balke[3] showed that protamine resembled the alloxuric bodies in that it gave a white precipitate with cuprous

[1] Kossel: *Zeitschr. f. physiol. Chem.*, Bd. 22, S. 178, 1896; and *Stzber. d. Preuss. Ak. d. Wiss.*, Bd. 18, S. 403, 1896.

[2] Hedin: *Ztschr. f. physiol. Chem.*, Bd. 22, S. 191, 1896.

[3] Balke: *Journ. f. prakt. Chem.*, Bd. 47, S. 537, 1893.

oxide from an alkaline solution. Thus, if hydroxylamine hydrochlorate is added to a solution of protamine and then a few drops of Fehling's solution, the cupric hydrate is reduced to the cuprous, and this gives a white precipitate with the protamine. Balke also showed one other very interesting reaction of protamine, for he found that it gave the biuret reaction. This has been confirmed by Miescher and by Kossel for salmin and sturin. As Kossel points out, sturin resembles the peptones in some respects, but differs from them in not yielding any of its nitrogen in the form of amido-acids. Protamine gives neither the xanthoproteic nor Millon's reaction. Kossel suggests that the biuret reaction in those cases where it occurs is due to the basic constituent of the proteid molecule. In a later paper Kossel[1] describes the preparation of a protamine from the spermatozoa of the herring. This one he names *clupeine*, and assigns to it the formula $C_{30}H_{57}N_{17}O_6$.

In his examination of the spermatozoa of *Arbacia* Mathews[2] prepared a protamine which he has termed *arbacine*, which shows differences from those previously examined. The sulphate was prepared according to the method employed by Kossel, and from this the free base was obtained. It gives an alkaline solution in water, gives the biuret reaction, and gives no precipitate with Millon's reagent, though the fluid turns red in boiling. Like the other protamines it precipitates proteids or Witte's peptone in alkaline solutions, but it again differs from them in the lower amount of nitrogen it contains. Mathews finds that in the spermatozoa it is combined with the nucleic acid; that there is no free nucleic acid present. The chromatin apparently consists entirely of arbacine nucleate. In examining the ripe spermatozoa of the herring he shows that the chromatin in all probability again consists entirely of clupeine nucleate, clupeine being the protamine which Kossel lately described as prepared from the herring. He further confirms the statements of Miescher and Kossel that

[1] Kossel: *Sitz. d. Ges. z. Beford. d. g. Naturwiss. in Marburg.* July, 1897.

[2] *Loc. cit.*

no protamine is to be obtained from the spermatozoa both of oxen and hogs. It is remarkable to find that the chromatin of such cells as the spermatozoa should chemically consist of substances which relatively to proteids are of simple constitution. If it be true that hereditary characteristics are transmitted by the chromatin of the reproductive cells we should have expected a most complex chemical structure for these parts, and it therefore becomes the more striking to note that the most complex protamine obtained, arbacin, is from the animal lowest in the scale, and that in the higher vertebrates examined no protamine is present at all.

THE FORMATION OF URIC ACID.

Following on his discovery of a group in the nuclein molecule, which yielded alloxur-bases, Kossel suggested that the nucleins might prove the source of the uric acid excreted by animals. The first direct evidence in favour of this view was, however, first supplied by Horbaczewski[1] who found that if spleen pulp be mixed with blood and a stream of air passed through it, on keeping this at body temperature for a few hours the xanthine bases gradually disappeared and uric acid appeared in their place. The experiments of giving the alloxur-bases as drugs to animals has not led to definite results in this direction, but in experiments with nuclein or nucleo-proteids the results in the case of most observers have been in the direction of a proportionate increase in the uric acid out-put. In a recent paper, Jerome[2] narrates the result of an experiment upon himself in which the uric acid excretion was determined upon different dietaries. He concludes that "the daily out-put of uric acid is so easily, so surely and so largely controlled by the use of suitable articles of diet as to make it highly probable that the variations in the amount of uric acid excreted in health from day to day are

[1] Horbaczewski: *Monatsh. f. Chem.*, Bd. 10, S. 624, 1889; and *Ztschr. f. Physiol. Chem.*, Bd. 18, S. 341, 1893.

[2] Jerome: *Jour. of Physiol.*, vol. 22, p. 146, 1897. (This paper contains an account of previous work upon this subject.)

chiefly due to the larger or smaller quantity of alloxur-holding bodies absorbed from the food: chiefly, but not wholly, for there are other influencing conditions the effects of which have yet to be worked out".

According to Horbaczewski, the production of uric acid after the ingestion of nucleins is not derived from the alloxur-bodies taken, but rather depends upon the leucocytosis which they induce, *i.e.*, that it is produced chiefly from the nuclein of the leucocytes as they break down. Thus he points out that the amount of uric acid excreted varies directly with the degree of leucocytosis which had immediately preceded it, so that a direct relation between the two seems to be definitely determined. Mares,[1] however, objects to this view that it is by no means proved that the uric acid results from disintegration of the leucocytes, but rather that it may arise from the metabolism or increase in rate of formation of the cells.

PHOSPHOCARNIC ACID.

In some respects allied to the nucleins is a phosphorus-containing acid obtained by Siegfried[2] from muscle and named by him phosphocarnic acid. This is contained in the watery extract from muscle and is obtained from it by first precipitating the phosphates by calcium chloride and ammonia, and then, while boiling, ferric chloride is gradually added, the reaction during this stage being kept weakly acid. In this way the iron salt of phosphocarnic acid, carniferrin, is precipitated and the constancy of the analyses of several preparations both of carniferrin and the free acid prepared from it proves that we are dealing with one body only, which is of constant composition. By boiling with dilute mineral acids phosphocarnic acid is split up into a carbohydrate which reduces Fehling's solution, carbonic, succinic, paralactic and phosphoric acids and in addition a new acid called *carnic* acid. The nature of the carbohydrate has

[1] Mares: *Wien. Sitzungsber*, Bd. 101, Abthg. iii., 1892.

[2] Siegfried: *Ber. d. d. Chem. Ges.*, Bd. 28, S. 515, 1895; and *Ztschr. f. physiol. chem.*, Bd. 21, S. 360, 1896.

not yet been determined. Carnic acid[1] is of some considerable interest. It is free from sulphur having a formula $C_{10}H_{15}N_3O_5$; it gives the biuret but not Millon's reaction. It forms an addition compound with one molecule of hydrochloric acid in which the chlorine is not precipitated by silver nitrate until it is boiled with nitric acid. It thus gives all the reactions of antipeptone with which Siegfried considers it identical, a view which is confirmed by Fränkel[2] who finds that antipeptone when prepared pure by the artificial digestion of proteids is sulphur free. Siegfried compares phosphocarnic acid to the nucleins, pointing out that where the nucleins yield proteid phosphocarnic acid yields antipeptone and he therefore suggests for it the name nucleon. Siegfried considers that the muscle-nucleon or some compound of it plays an important part in the metabolism of muscle during its activity, for he finds that its amount is much less in fatigued than in resting muscle and points out that the formation of carbonic acid, phosphoric acid, and lactic acid during a muscle's activity can be at once explained as the result of the decomposition of phosphocarnic acid. This view is further strengthened by the experiments of Krüger[3] who shows that muscle-nucleon on hydrolysis gives off considerable quantities of carbonic acid and that no other extract from muscle does this. For this production of carbonic acid no oxygen is required.

Phosphocarnic acid has also been prepared from the urine[4] where it is present in very small quantities and also in milk.[5] The nucleon obtained from the latter source is however a slightly different structure, for it yields on decomposition fermentation lactic acid in the place of paralactic. From the fact that it yields antipeptone on decomposition

[1] Siegfried: *Ber. d. k. Sächs. Ges. d. Wiss. zu. Leipzig. Math.-Phys. Classe.*, 1893, S. 485; *Arch. f.* (*Anat. u.*) *Physiol.*, 1894, S. 401, and *Ber. d. d. chem. Ges.*, Bd. 27, S. 2762, 1895.

[2] Fränkel: *Wiener. Med. Blätt.*, 1896, S. 703.

[3] Krüger: *Ztschr. f. physiol. Chem.*, Bd. 22, S. 95, 1896.

[4] Rockwood: *Arch. f.* (*Anat. u.*) *Physiol.*, 1895, S. 1.

[5] Siegfried: *Loc. cit.* and *Ztschr. f. physiol. chem.*, Bd. 22, S. 575, 1896. Wittmaack, *ibid.*, Bd. 22, S. 567.

with dilute acids and that it offers a possible explanation of the production of some of the waste products of muscular activity, muscle-nucleon becomes a body of considerable importance and interest, though fresh confirmatory work is required before we can consider its *rôle* as a store of muscular potential energy at all satisfactorily determined. One fact which seems to bear against this view is that the amount of it present in muscle is very small, *viz.*, from 0·1 to 0·2 per cent. Its iron ccmpound carniferrin has been shown by Hall[1] to be readily absorbed in the small intestine. It may thus to some extent serve as one means by which iron is taken into the body.

T. G. Brodie.

[1] Hall: *Arch. f.* (*Anat. u.*) *Physiol.*, 1894, S. 455.

JULIUS SACHS.[1]

Si quis tota die currens
Pervenit ad vesperam satis est.

AFTER great suffering Julius Sachs sank peacefully to rest at six o'clock on the morning of 29th May, 1897, at Würzburg, the scene for many years of his labours. Wherever scientific botany has a home, and by many outside the narrow circle of specialists, this loss has been regarded as irreparable. By no one has it been felt more keenly than by the writer of these lines, who will always thankfully recall the happiness it has been to him to have been closely connected throughout a long series of years as pupil and friend with him who has passed from our midst.

When I attempt to briefly sketch the life of the man to whose brilliant intellect botany is so greatly indebted, there rises involuntarily to my mind the saying of Petrarch's that I have quoted above, a saying at once so sad and yet so consoling.

Yes, his life was a struggle, a ceaseless, single-minded pressing-forward without rest to the goal of knowledge. To him study, research, teaching were not merely the external activities of his calling that might be laid aside for hours, days or even weeks, and then be again resumed. They absorbed his whole being more than was good for his personal welfare. But the evening came after this long day in which he had so faithfully laboured. No one realised this more fully than he himself. A prey to physical suffering, his sharpest pang was that he could no longer work for science with his former energy, and if anything made it hard for him to face death, it was the knowledge that he must leave behind as an unfinished sketch much that he wanted to say to the world.

[1] This article, which is somewhat shortened, is a translation by Miss E. D. Shipley of Professor Goebel's article in *Flora oder Allgemeine Botanische Zeitung*, 1897.

He had been chiefly occupied during these last years with a work which, under the title of *Principien Vegetabilischer Gestaltung* (*Principles of Vegetable Form*), was to set forth his views upon causal morphology. "I should feel it an immense grief if I were prevented from writing this book," he says: "it would embody the thought of forty years, and it is always important that one's ideas should be long and thoroughly brooded over. To finish it would render the last years of my truly miserable existence in some degree bearable."[1]

We shall refer again to the purposes of this book, and turn now to a short account of Sachs' career.

He was essentially a "self-made man," who found it by no means a light matter to attain the eminence which led the most distinguished German universities each to desire to win him for itself. The story of his early years as it appears in these pages is taken from an autobiography intended for his own family, Fräulein M. Sachs having kindly made extracts from it for my use. It will be of great interest to many who only knew him as a mature man occupying an honourable position to learn how literally true were the words "tota die currens".

Sachs was born on 2nd October, 1832, at Breslau, where his father was an engraver. For a time his parents lived in the country, and this may have contributed to the early awakening of his mind to the beauty of nature at which he always looked as much with the eye of an artist as with that of an observer. The design that he cherished at one time of writing a work on the beauties of the plant-world was unfortunately never realised. It would have been of the greatest interest if he, an adept in the art of word-painting, an enemy to all affectations and mannerisms, had given us his thoughts upon this theme.

His first experiences of school life were not pleasant. Learning by heart, that purely mechanical acquisition of knowledge, was a burden to him as it has been to many another highly gifted scholar. Of much greater importance

[1] The quotations are principally taken from letters.

than his school instruction was his father's training in drawing. From his thirteenth to his sixteenth year he drew and painted flowers, fungi, and other natural objects, and his artistic talents played, as we shall see later, an important *rôle* in his career.

His family possessed but few books and the boy felt stirring within him a longing, doubtless inexplicable to himself, for intellectual advantages. And thus his brother's acquaintance with the sons of the physiologist, Purkynje,[1] at that time a professor at Breslau, was of great importance to him. His brother brought home the *Penny Magazine* from these playfellows, and the prehistoric animals depicted in it aroused so great an interest in Julius, then as always thirsting for knowledge, that although he could not understand the English text the "extinct monsters" appeared to him most realistically in his dreams! Later he himself came to know Purkynje's sons and this acquaintance shed a ray of light upon his life; for the first time he saw a refined home, free from all petty cares as to daily bread, filled by stirring intellectual life, and dominated in every detail by the imposing figure of the white-haired professor who inspired Sachs with the greatest respect. Julius learned to press plants from his sisters and heard that there were such things as botanical collections: he proceeded to start one for himself. His father, who knew the popular names of many plants, encouraged these endeavours. They made expeditions in the early morning hours, and at fourteen years old Sachs could already determine his plants according to Scholtz's *Flora*. But his herbarium was stolen, and this was his first bitter, deeply felt grief. He related his loss to every one and could not understand that other people failed to recognise its gravity. He never again collected plants until in later years, as professor, he started an herbarium for the purposes of demonstration. The way in which at the present day so many botanists entirely neglect

[1] J. E. Purkynje (1787-1869) was Professor of Physiology and Pathology in Breslau from 1823 till 1850, and afterwards in Prague. He was the author also of a botanical treatise (*De cellulis antherarum fibrosis nec non granorum pollinarium formis commentatio phytotomica*, Breslau, 1830).

the practical knowledge of plants was wholly distasteful to him, as the following remark in one of his letters shows. "I strongly disapprove of the so-called 'physiologists,' to whom the commonest meadow and garden flowers are unknown, especially as such people generally have but little knowledge of physics." And if he complained many a time in joke of the foolishly unnecessary and tedious multiplication of phanerogamic varieties he was far from undervaluing the knowledge and study of them. Indeed we shall come across instances of the keen interest in the common problems of systematic botany which constantly appears in his writings.

It was his mother who conceived the thought of allowing him to attend the gymnasium, a privilege accorded to none of his brothers, for this considering the family poverty involved no slight risk.

The years he spent at the Elizabeth Gymnasium formed a bright picture in Sachs' life. The school work was congenial to him, it lifted him out of the petty surroundings of his home-life into a higher sphere. He attended the gymnasium from 1845 to 1850. Of the masters only one —Dr. Rumpelt—came at all into personal contact with him. He recognised Sachs' exceptional talents and the two became good friends. On the other hand the natural science master, the lichenologist Körber, only repelled him. Körber could not instruct and had no conception how to impart anything worth knowing about his subject. Sachs therefore worked on at his scientific pursuits unaided and undirected. He read eagerly, without its doing him any harm, Oken's *Philosophy of Nature* which he had bought at a sale for a few pence, began to make a collection of skulls, and wrote a monograph on the crayfish. Körber's attention was drawn to this work by Dr. Rumpelt; he sent for Sachs and solemnly warned him against devoting himself to natural science, on the ground that it would not bring him in a half-penny! One cannot but rejoice that this advice was not acted upon.

In the year 1848 Sachs lost his father, and, in the following year, his mother. Thus orphaned, he lived at first with his brother, where, to his great joy, he was

allotted a room in the roof which, although otherwise unattractive, afforded him the opportunity of carrying on his scientific studies in his scanty leisure. Here, for instance, he mastered the Latin Anatomy of Bartholinus. It became more and more imperative however that he should face his position. He left the school (having risen to the upper second form) and wished to go to sea.

In the meantime Purkynje had been called to Prague. He remembered his son's friend and wrote suggesting that Sachs should come to him as a kind of private assistant. He was to prepare natural science drawings and in return to receive the modest salary of 100 florins a year and his keep.

After numerous difficulties with his guardians, Sachs left Breslau on 14th February, 1851, for Prague. He found there shelter, it is true, but no home. Purkynje was a man of high attainments, for whose genius Sachs had great respect. But their peculiar temperaments made it impossible for them to understand each other, and the elder naturalist had no word of recognition, sympathy or encouragement for the younger. He was of peasant origin and this stuck to him all his life. Sachs, on the other hand, felt himself—as he said with reason, in spite of the reduced circumstances of his family—to be a born aristocrat, and so there could not fail to be friction between them.

Whilst Sachs was at Prague, the question arose whether he should remain simply an illustrator of scientific writings or should carry on his studies further. Fortunately he decided upon the latter course, and despite the time that had elapsed since he left school, successfully passed his matriculations at Prague in the autumn of 1851 with a view to entering that university.

The young student was already too independent and critical to be an ardent frequenter of the lecture room, where it would have required a man of exceptional ability to have secured his attendance, and it was evident that there were at that time but very few such men at the University of Prague. Botany was represented by Kosteletzky, who was lecturing upon Schleiden's works. Sachs attended two or

three lectures and then stayed away; the truth was that he needed no teaching on this subject. He paid special attention to chemistry, physics and mathematics. But the only man who attracted and helped him on was Robert Zimmermann,[1] who invited him to his house. "I went to him with an inclination towards philosophy but he directed me into the right way," Sachs says, speaking of Zimmermann; "he and any earlier teacher Rumpelt are the only two who gave me any real help, apart from their aid I am self-taught". He read a good deal of philosophy after he had become acquainted with Zimmermann,—Herbart, Leibnitz, Kant, Locke, Hume and even the Schoolmen. At the same time he was privately working at zoology and botany, and for several years paid special attention to physics and mathematics. In 1856 he was made Doctor of Philosophy, a degree which at that time was hard to obtain at Prague. His outward circumstances, since he had separated from Purkynje, remained precarious; he earned small sums by literary work, drawings of fossils, etc., and at this time made his first experiments in the physiology of plants. In 1857 he was made Privat-docent in Plant Physiology. Up to that time this had not been a recognised subject and there were various difficulties to overcome. "Two lectures are ample for all there is to say upon the physiology of plants" said Rochleder, the chemist, and at that time he was not so very far wrong.

Sachs, who later was certainly the best teacher that the new botany has produced, was by no means a success as Privat-docent. One reason of this may be that he took but slight interest in the art of teaching. He lived wholly for science and was beyond measure studious, "it engrossed my thoughts even when I was out walking," he says. This being so, it came to him, according to his own account, more or less as a revelation that what he had to do was not only to acquire as much knowledge as possible but also to produce some original work. From that time he only

[1] Robert A. Zimmermann, born at Prague, in 1824, studied philosophy, mathematics and natural science, became Professor of Philosophy at Prague in 1852 and since 1861 has held the same chair at Vienna.

sought to work out his own ideas, to attain his own aims. He became acquainted with several of the chief exponents of botany of the day, such as Unger, Nägeli, and Alexander Braun, all of whom he met at the Natural Science Congress in 1856 at Vienna; and also about 1857 with Hofmeister who, in the intercourse that lasted between them for many years, influenced Sachs strongly, though, as the latter considered, at times in such a way as to perplex him.

In the meanwhile he was finding his life in Prague almost unbearable. The patriotic Czechs of the National party opposed him as a German, and openly told him that they wanted to drive him away. Whilst this was going on, the attention of Prof. Stein, the well-known zoologist, had been directed to Sachs. Stein had formerly devoted some of his time and energy to the Academy of Forestry at Tharandt and introduced Sachs to the chemist Stöckhardt, the director of this institution. Sachs was invited to draw up a statement as to the relation of plant-physiology to agriculture, with the result that he was called to Tharandt as physiological assistant in 1859. He went there in the March of that year. His chief work here was to show that land plants could be raised in aqueous solutions of nutrient salts, but he was busy at the same time with other physiological experiments. "Die entdeckungen lagen damals am Wege" was his opinion, "die Botaniker trieben audere Dinge". Even then Nägeli, for instance, described Sachs' researches as belonging to the chemistry of agriculture, there was as yet no talk in Germany of the chemistry of plant-physiology.

In summer he started work at four o'clock in the morning, and by so doing found time during the years 1859 and 1860 to study the earlier plant physiologists besides doing his own work. These literary studies caused him in 1860 to suggest to Hofmeister that they should edit a large hand-book of botany, in which the collected results of what we now call "general" botany should be critically set forth. The *Handbuch der Physiologischen Botanik* remains, as is well known, a fragment: various collaborators who had undertaken certain parts drew back,

and Hofmeister fell ill and died in 1877 without being able to complete his share; but in spite of all mishaps the four volumes that appeared rank among the most valuable productions of more recent botanical literature. Sachs had to frequently give addresses at agricultural meetings and so gained the useful knowledge that he had a natural gift for public speaking.

In the winter of 1860-1 he was invited to become the head of the recently established agricultural department of the polytechnic at Chemnitz. His position there bristled with difficulties, and he welcomed the proposal that he should accept the Chair of Botany and Natural History at Poppelsdorf near Bonn, whither he removed in 1861. Here he married and in time became the father of two daughters and a son.

As regards science the six years spent at Bonn are amongst his most fruitful. Besides a number of other works it was here that his *Experimental Physiology* was written and the *Text-book* begun. His lectures were highly appreciated and at the end of two years he was relieved from lecturing upon mineralogy and zoology; henceforward he dealt only with physiology during the winter, and in the summer delivered special lectures on agricultural plants. There was but little intercourse between him and the botanist Schacht, who was then at Bonn, but who was already in bad health, and whose temperament was thoroughly uncongenial to his own. With Schacht's successor, Hanstein, on the contrary, friendly relations ensued. On New Year's Eve, 1866, he received the news that he had been called to Freiburg im Breisgau as successor to De Bary: he went there in April, 1867. A small salary and a poor garden formed two undesirable elements in his life at Freiburg, and after three terms he willingly left to go to Würzburg. There, as we know, he remained, in spite of brilliant offers to move elsewhere. As early as 1869 he received a call to Jena, in 1872 to Heidelberg, in 1873 to Vienna, in 1877 to Berlin, where later they tried to obtain him for the Agricultural College; he was also invited to Bonn under tempting circumstances. When

Nägeli retired, the professorial chair at Munich was offered him. It is much to be regretted that he did not accept one of these invitations whilst his health was still good, especially as the climate of Würzburg is hardly favourable to nervous constitutions. It may perhaps have been the needs of his family which pressed heavily upon him, or attachment to all he had acquired at Würzburg and dislike to the loss of time and strength inseparable from each change of place, that kept him there. The Government testified its appreciation by investing him with titles and orders, as early as the autumn of 1871 his colleagues chose him for their rector, and he was repeatedly elected to the Senate.

With the commencement of his professorial life at Würzburg, Sachs' "Wanderjahre" came to an end. They had been, as the preceding facts show, beset with difficulties. "I was thirty-six years old when, with a salary of about 2000 guldens I came to Würzburg and found a hole in which to hide my head. During the three previous years in which I had laid aside the *Experimental Physiology* and had been writing the *Text-book*, I had had a severe struggle in the strictest sense of the word to provide for the wants of my family. I was thirty-seven years old when I succeeded for the first time in investing 200 thalers in the public funds, and had for twenty years daily worked from fourteen to fifteen hours. As you see, my life has not been an easy one, and yet I wish that things went as well with me now as they did then, for what I have been through since is truly more than a man can bear."

The strong expression that he uses in speaking of the laboratory at Würzburg shows that there was much to be desired both in it and in the gardens attached to it. The laboratory which under his direction obtained a world-wide reputation and attracted young botanists from all parts, was housed, together with the clinical schools and the Institute of Pharmacology, in a building that contrasts most modestly with the handsome modern structures that have arisen in many universities. And yet how much he accomplished in it! Little by little the whole of it came to be given up to botanical purposes, Sachs being much too modest to insist

on a new botanical laboratory in spite of the fine new buildings that were erected for the other sciences. He contented himself with the addition of a very beautiful and suitable lecture-room. He was particularly anxious about the garden, which was laid out on barren soil made up chiefly out of the rubbish-heap of an old fortress *glacis.* He gave it his own personal and devoted attention, and was rewarded by a luxuriant vegetation where formerly there had been but a barren waste. Later on he divided off a small part of the garden for special purposes and this he attended to himself with the help of his laboratory servant. There he made open-air experiments, and there also was the well-known *Schilderhaus* (sentry-box) for experiments in etiolation, etc. The cultivation of strong, healthy plants for the purposes of investigation was in his opinion an essential part of experimental physiological work; he excelled in the art and deemed it worthy of individual, personal attention. There were almost invariably plants growing in his work-room, but in summer time, when growth was going on in the plant-world, it was essential to him to make constant observations out of doors and to meditate upon his investigations as he strolled about the garden.

The astonishing amount of work that he managed to get through from his earliest days could not but affect his constitution. He said himself that he had paid for each of his books with wearisome ill-health, and even the strongest nerves could not stand such ceaseless labour. Added to this came his wife's long tedious illness which undoubtedly helped to undermine his strength.

Bearing these facts in mind it is perhaps more possible to form a just estimate of his relations with the outer world The latter part of his life found him a lonely man who had estranged many of his friends by bitter and sometimes even unjust criticisms. We shall perhaps condone his trenchant animadversions upon the botanical writings of his day if we remember how his sensitive, highly strung temperament must have suffered at times from the irritation of private affairs. And then again, science represented to him all that is highest in life, and it followed that any work which

he considered bad from a scientific point of view seemed to him a crime. More than this, much that appeared of great importance to others had no weight with one who regarded the mission of science from so high a standpoint and whose refined nature could not fail to despise all ambiguity, empty phrases and affectations in its literature. He considered the great defect in this to be that, whilst each isolated investigation is deemed a personal achievement and quoted as such, important generalisations were regarded as impersonal property. He was by no means a man who could not endure contradiction and was always ready to listen to it when well founded; it was only when the opposition seemed to arise from incapacity and stupidity that he was roused to fierce anger. His standpoint is best described in the following words written to a friend at the end of a keen discussion: "After all, in science as in ordinary life, all hinges upon whether a man accept the general point of view of his opponent; when that is done it is always possible to arrive at some satisfactory conclusion, and I hope this will always be the case with us".

Although the purely intellectual side of his nature outweighed the emotional, he was invariably grateful for the smallest services, and to me he always proved an indulgent, lovable teacher. At the same time he could coldly repel all who were uncongenial to him. He agreed with Goethe "Sage nur von deinen Feinden, warum willst du gar nicht wissen," etc.

As time went on he became more and more dissatisfied with the state of botanical literature. Such dissatisfaction however did not keep him from incessant toil whenever he was well enough and more especially when the sun shone. Like Goethe and many other sensitive natures, he was strongly affected by sunshine or the lack of it. "If you imagine yourself transplanted from Java to Bavaria and that the sun's face has been veiled for the last three weeks by a layer of sail-cloth 100 metres thick, you may form some conception of the vegetation in our garden. The grass and leaves grow as though this were a dairy-farm! Every

one is charmed with our luxuriant vegetation, but there are no signs of blossoms. It is as dark at four o'clock as it would be at the same hour at Christmas, and it has been like this for the last three weeks. I should not complain, liking as I do to take things as they come, but unfortunately I cannot live without sunshine and the lack of it makes me ill."

It was at Würzburg that Sachs first found fit opportunity to develop his talent for teaching. Too often it happens in lecture-rooms that "*man viele sieht, die nicht da sind*" but this did not apply to him. His fascinating, lucid expositions stimulated the students, whilst he knew well how to practically illustrate his subject. He worked incessantly at the materials for demonstrating, drew and painted a number of diagrams, and was constantly adding to his stock of dried plants, alcohol preparations, models and cultures. He considered that all should be in due relation to the subject matter in a scientific lecture as in the acting of a play. In the winter he lectured on general botany (anatomy and physiology) and in the summer on the "Natural History of the Plant World". Besides this he often gave experimental demonstrations in the summer and this necessitated a great deal of work; occasionally he lectured on the history of botany and on the physiological basis of morphology. After 1874 he had a class every term for microscope work.

A great number of botanists worked at one time or another in his laboratory. The first were Gr. Kraus and Millardet (both formerly at Bonn and Freiburg). Among others attracted by him to Würzburg were, Baranetzky, Brefeld, Francis Darwin, Detlefsen, Elfving, W. Gardiner, Godlewski, Goebel, Hansen, Hauptfleish, Klebs, H. Müller-Thurgau, Moll, Noll, Pedersen, Pfeffer, Prantl, Reinke, D. H. Scott, Stahl, Vines, De Vries, Marshall Ward, Weber, Wortmann, and Zimmermann. He insisted upon his pupils being in earnest about science, and he brooked no laziness. Weak natures naturally felt his influence most strongly, but he set a higher value on those from he could gain something.

With failing health he withdrew more and more into

himself: "I am beginning to take private pupils again," he writes, "but there is little pleasure in it. When a professor reaches the age of sixty, he ought *eo ipso* to be pensioned off with his full salary; it might be possible to arrange a university that would serve as an almshouse but I would not go into it."

He urged his pupils to make comprehensive studies even as he was constantly striving after wide generalisations. He was a master in the art. We have only to think of his *Experimental Physiology*, his *Text-book* in four editions, his *History of Botany* and his *Lectures on the Physiology of Plants*. Although he wrote with ease, he bestowed great care upon composition, and usually made several rough sketches before the work was done to his satisfaction. In later years he generally dictated, and the *Lectures* were written in this way. The great debt owed by modern botany to his *Text-book* can scarcely be appreciated even yet by the younger generation of botanists.

No entirely satisfactory text-book had appeared since Schleiden's *Outlines*, a book that contained much that was critically suggestive, but, on the other hand, was one-sided and tinged by the author's personal prejudices; nor had the later editions of it been brought up to date with the advance of science. Sachs' book was the first to make Nägeli's and Hofmeister's researches known to the world. It was written in an unusually clear, literary style, and contained all that was best according to "the present state of science" as the title-page says, especially the author's important physiological researches. The letter-press was interspersed with numerous illustrations, chiefly Sachs' own work and not seldom the results of laborious, tedious experiments. These illustrations have been frequently reproduced and, contrary to Sachs' express wish, have become common property. Too often it has been considered quite unnecessary to obtain his consent to the use of the figures, and the appearance of a newer text-book decked out with his own illustrations elicited from him the somewhat bitter though just remark, that a student, using this book, would

surely think that he (Sachs) was employed by the author to illustrate his work. Towards the end of his life the frequent revisions needed for a text-book became a burden to him: he could not make up his mind to a fifth edition and wrote his *Lectures* in a freer style of exposition.

The book, however, that presents the best insight into Sachs' individuality is his *History of Botany*. Nägeli had originally been commissioned to undertake this work, which was to form a part of *The History of Science in Germany* issued by the Royal Academy of Bavaria, but he had soon abandoned the task. It cost Sachs five years' continuous toil. As with all human work it has many defects and omissions, but the lucidity, the profound philosophical bent of Sachs' mind, lend an incomparable charm to the whole. An English translation of this work appeared in 1890.

If I further attempt to briefly characterise Sachs' importance with respect to science it is with a due sense of the difficulties of the case. His activity was so comprehensive, the results of his researches have become through his *Text-book* so largely common property, that it is not easy to briefly set forth what he has done for science. One would have to write a history of botany from 1860 onwards to justly rate his services. But this is by no means the place for such a work, nor do I feel equal to the task. The extracts already given show that he was no one-sided physiologist, and he was fully aware of the fact. "It may surprise you," he writes, "that from my boyhood the mysteries of relationship (systematic botany) have interested me more than those of biology and physiology. I have apparently specialised in the last-named branch of science, because I have always been of the opinion that the ultimate problems of systematic botany can only be solved by physiological methods." His latest treatises most clearly reveal what he meant.

De Bary's remarks with respect to Mohl apply more or less to almost all distinguished investigators (*Bot. Zeitung*, 1872, p. 572). "As regards a number of discoveries for which we are indebted to Mohl, his claims to

priority in them may justly be disputed if this expression be taken to denote the pretension to have first seen or spoken of a thing, . . . the lucid, confident recognition of it is however due to Mohl's observation." But in Sachs' case the remark applies not merely to the observation of facts, to which Mohl confined himself, but to the bringing into prominence the importance of such facts in their relation to the common stock of our knowledge, and to the right ordering of observations in the general building of knowledge; work on which he laid great stress. He writes: "As I read your book I feel anew how much more merit there is in working out a comprehensive subject from reliable sources, and from a higher standpoint, than in constantly supplying fresh contributions, which, however meritorious in themselves, are yet as the scattered stones of the hillside compared to milestones pointing us on our way!"

Sachs is best known and most famous as the founder of the modern physiology of plants, and his physiological works may be next touched upon. "My earliest treatises," he once wrote, "were composed at a time when the physiology of plants was simply non-existent; I myself was entirely self-taught and consequently much of my work was imperfect, especially the manner of exposition." Nevertheless these earlier works are of great importance. Next to be named come his works upon chemical philosophy. The investigations of Ingenhouss, Th. de Saussure, Liebig, Boussingault and others had supplied the foundation upon which, in connection with the results of plant-anatomy, a more exact knowledge of the phenomena of metabolism was to be built up. It was Sachs who first pointed out "that the starch in chlorophyll is not merely a secondary deposit but must be regarded as the product of the assimilating activity (produced by the action of light) of the granular, chlorophyll substance: that it is formed in the chlorophyll out of its original elements and is conducted to the growing buds and to the tissues which store up the reserve material"[1]: a brilliant addition to our knowledge, the

[1] *Collected Essays*, p. 335.

fundamental importance of which needs hardly to be demonstrated at the present day.

The formation of starch largely engaged his attention later on. He contrived a simple means of quantitatively estimating starch-assimilation, and by the application of the "iodine test" to leaves or portions of leaves respectively, supplied an extraordinarily simple and instructive method of demonstration.

His services in improving the culture of plants in nutrient solutions are well known. They drew down upon him a violent attack from Knop which deeply wounded him, and not without reason. It is now one of the most elementary experiments in the physiology of plants to rear a plant from germination to seed-bearing by the administration of nutrient salts; but at that time it was maintained that the seed-bearing plants of maize must have been placed in the solution of nutrient salts after they had attained a flourishing condition!

He incidentally discovered the interesting fact that polished marble slabs may be corroded by roots; a fact of some importance for the understanding of the functions of these organs. He began to work upon entirely virgin soil when, at the close of his fiftieth year, he set on foot investigations which brought to light by microscopical tests, and above all by microchemical methods, the movements, chemical changes, and final consumption of the reserve material during the growth of organs. These experiments have also proved of fundamental importance, and he lays stress upon the fact that they served to first lead him to think that the chlorophyll grains are the true organs of assimilation. A bare reference must suffice to the classical treatises on the germination of the date-palm, of grasses, or on inulin, etc.

In later years he ceased to contribute experimentally to our knowledge of metabolism. Other problems had meanwhile claimed his attention. His investigations—the first to be made—into the action of heat claim special notice. The phenomena of freezing had long been in need of investigation, and here also Sachs' work created a clear conception

of the problem and went far towards clearing it up. Even more important were "The Physiological Experiments upon the Dependence of Germination on the Temperature". For by these the law of the "drei Kardinalpunkte" (three cardinal points) was established, and the term "Optimum" introduced for one of them—a name that has been adopted in other departments of science. These experiments were carried out with the simplest appliances, not even in a botanical laboratory, but in his own rooms at Prague. His great manual dexterity and skill in devising simple, but extremely effective, instruments were most useful to him.

The discovery that with sensitive organs there are temporary conditions of rigor due to heat and cold has become an intrinsic part of physiology, whilst the establishment of the fact that not only light, but at the same time a sufficiently high temperature is needed for the formation of chlorophyll in the higher plants, was of great interest.

From amongst the series of researches grouped together in the *Gesammelten Abhandlungen* (*Collected Essays*) under the heading "The Action of Light" I should like shortly to refer to the treatise "Upon the Influence of Daylight on the Production and Development of Different Plant-organs".

The fact that the formation of cells and organs is dependent upon light was submitted in this paper for the first time to a searching investigation; it was shown that the formation of roots was in many cases directly favoured by light; the conclusion was drawn from Wigand's data that with fern prothallia light determines the dorsiventrality, and the phenomena of etiolation, which still present many enigmas, were more closely examined. The investigation into the action of light through the medium of the foliage-leaves upon the formation of flowers, was especially important to Sachs, because it formed the starting-point for his later theory of "Matter and Form". It showed him that plants such as *Tropaeolum*, *Brassica*, etc., continue to produce etiolated stem-parts and leaves in darkness "in sufficient quantity for the production of fresh blooms if this depended only

upon the bulk of the material stored for the purpose and not also upon the particular quality of it," a fact that later led him to form his theory as to the specific matter out of which organs are formed. The formation of blossoms was proved to depend directly or indirectly upon light, inasmuch as by the assimilating activity of the leaves in light, the materials destined to produce flowers are formed. Later research into "The action of the ultra-violet rays upon the production of flowers" seeks to define this phenomenon more closely.

The action of coloured light upon plants in respect to assimilation and to their heliotropic curves, etc., received soon after valuable confirmation. Sachs introduced the simple and convenient method of counting the bubbles given off by water-plants in light, and came to the conclusion (which lately has again been questioned) that the so-called chemical rays have very little to do with the giving off of oxygen.

A keen controversy was aroused by the opinions he formed in consequence of his researches into "The movements of water in plants". But even if his inbibition theory be rejected it must not be forgotten how many valuable facts are due to his activity in this field. The effects produced by the chemical and physical state of the soil upon transpiration, the checking action of salt solutions, low temperatures, etc., were well established; the "Lithium Method" was used for measuring the rate of the transpiration current; and the profound and far-reaching importance of transpiration for the life of most plants was demonstrated.

A further laborious and protracted series of experiments dealt with the phenomena of growth and of movements produced by stimuli. Among the more notable of these are the construction of the first auxanometer, the graphic description of his observations, and the recognition of the grand period of growth. His investigations into the growth of the main and side roots first proved convincingly the factors which condition the regular extension of the root-system in the ground, and established the distribution of growth in roots, as well as the correlation between main and

side roots. A number of isolated observations are also to be found in this exhaustive treatise. Sachs' clear, perspicuous style renders it a pleasure to read any of his essays, even when he is compelled to enter minutely into detail.

The phenomenon of "Hydrotropismus" (the name originated with Sachs) had already been occasionally investigated, but Sachs showed it to be due to irritability, demonstrated its importance and facilitated the examination of it by a simple apparatus. The "Hängende Sieb" (hanging sieve) is now to be found, like the auxanometer and the klinostat, in every botanical laboratory.

The "Tropisms" (Heliotropism, Geotropism, etc.) made large demands upon his time and attention. When under Hofmeister's influence, as regards experimental physiology, he inclined to an external, mechanical conception of these, but abandoned this later. His own words best denote his standpoint: "I too should have nothing to say against the term "Lebenskraft" (vital force) and have indicated as much from time to time in my *History of Botany*, but the word has been spoilt and rendered nugatory by misuse. I say, therefore, to denote my conception of the organic world, that the province of true physiology begins where that of mechanics, physics and chemistry of organisms ends. Indeed, I go farther and maintain that the time will come when in physiology will be found the ultimate basis (what Goethe speaks of as "die Mütter") of all natural sciences. There is no need to say that this vitalistic view did not prevent him from working out with the deepest interest the phenomena of growth-curvatures. He also established the phenomenon known as "after-effects," and contributed many other valuable isolated experiments.

If he attached great importance to theories, he was fully conscious of their transitory nature; and I might mention as an example of this, that in his later years he did not lay so much stress upon his theory of Heliotropism. There will be more to say about this when reference is made to his treatise on orthotropic and plagiotropic organs.

In the meanwhile attention must be directed to the

essays upon the connection between cell-formation and growth, which in my opinion belong to his most brilliant achievements. As a result of Nägeli's researches on the apical cell, numerous botanical works had arisen dealing with the laws of cell-division. It was this tendency, exaggerated until it was justly dubbed "zellfängerei," that led men to neglect plants and organs as a whole for the mere cells, and to take it as granted that growth is determined by the manner and method of cell-division, much as the shape of a building is determined by the way the building-stones are laid one upon another.

Hofmeister's brilliant, though hardly well-grounded, opposition had but little success: only a few botanists took any notice of it. It was Sachs who, in his usual clear manner and by the aid of simple contrivances, first explained the relations between cell-disposition and growth. In his opinion, the latter is the determining factor, the arrangement of cells depending upon growth. This explained why, for instance, cross-sections through cylindrical masses of cells in plants belonging to widely separated groups may present the same appearance of cell-arrangements, as a developing alga or a hair of a dicotyledon. The introduction of the terms "anticlinal" and "periclinal" made a brief, striking bird's-eye view of the matter possible, and facilitated further study of the changes in cell-disposition occurring during growth. A large group of facts was brought together under a common heading; and not only was the way made smooth for further investigations into the causes of the arrangement of cells, but an important point of departure was also made for experiments on the evolution of organs which do not possess an apical cell.

The changes, which had gradually taken place in the cell theory, have led to an entire alteration in its original meaning. This prompted Sachs, who always felt the need of clear and consequently historically correct conceptions, to introduce the definition "*Energid*". In my opinion he thereby rendered good service to science. It was a great satisfaction to him that his achievements found favour with the most eminent histologists (Kupffer for instance), and

this consoled him for the fact that the botanists, now as on other occasions, instead of testing the innovation in its general application, sought only too zealously for instances in which it did not apply. But the time will surely come when it will be deemed absurd to describe a *Caulerpa*, for instance, as a "unicellular" plant, and it fell to Sachs to fit scientific nomenclature to recent advances in knowledge. It was self-evident to him that definitions are only a means towards generalisation and that they have absolutely no validity in themselves.

The essay upon orthotropic and plagiotropic plant-parts takes us into a region that lay nearest to Sachs' heart during the last years of his life, namely, that of physiological or causative morphology. In this treatise he deals with the connection between the structure (in the widest sense of the word) and the direction of the organs. The definitions "orthotropic" and "plagiotropic" were introduced, and referred more particularly to the dorsiventral structures that had long been neglected under the supremacy of the "spiral theory". He does not merely treat of the purely structural conditions, but of the causative relations between orthotropic growth and dorsiventral structure. Sachs would, I believe, have altered later his theoretical conclusions upon plagiotropism; they are based upon ideas which he no longer held, as we may see in the text, to be as thoroughly warranted as formerly. But putting aside these points, about which opinions still differ, we find ideas in this essay that are still working with considerable effect in morphology.

As a morphologist Sachs' activity displayed itself in one direction by some special studies that date from his earlier years, in another by his text-books, and again by his final general essays.

His two treatises on Collema[1] and Crucibulum, show him at work in the region of cryptogams. It was he who in his *Text-book* defended Schwendener's Lichen theory

[1] In this essay he approached very closely to the later lichen theory, when he said that it looked as if a parasitical fungus had established itself in the nostoc; he believed that the nostoc-heterocysts might develop into a mycellium.

at a time when the cautious De Bary (in his criticisms of the second edition of the *Text-book*) looked askance at it. The Archegoniates are treated in the *Text-book* with special interest, forming part, as they had done, of his own researches. His grouping of thallophytes (in the fourth edition of the *Text-book*), which met with such adverse criticism, has at any rate attained the satisfactory position of being approached again in our own days by many writers.

Throughout his life he cared little for those details that often fill men's lives, and preferred to view matters from a wide and general standpoint. In the first edition of his *Text-book* he had set his face against "idealistic morphology" at a time when it was dominant, and in a paragraph of his *History* that promises to become classical he laid bare the foundations upon which this tendency rested.

Darwinism was another bugbear to him and he intended to attack it vigorously in the *Principles*. "As far as it goes I am delighted to be free from 'the immutability of species' and to be able on good grounds to accept evolution. But it is absolutely uncertain *how* we are to conceive of this latter. Therefore I say that the natural system of classification is only to be explained by Descent, but how *this* is to be explained no one knows. I regard Descent as a fact, like gravitation, about which also we are absolutely in the dark." His whole conception of the world rebelled against "the crude materialism" which he thought he found in Darwinism; "if my *Principles* do not meet with the response I had expected, they have done me good service in showing me that Darwinism as a whole is entirely superfluous for any scheme of the final causes of nature. A superfluous theory has received its sentence."

He sought however to obtain some similar conception of causes by his theory of "organ-forming matter," which caused the external diversity of organs to appear dependent upon their material differences of substance, a view which had its origin in the researches alluded to above on the dependence of bud-formation upon the assimilation activity of the leaves. By this a theoretical basis was gained for

experimental morphology; deformities, galls, etc., could be referred to definite changes of substance; and the assumption that stem-forming substances find their way to the point of stem-growth, root-forming to that of the root-system, explained to him most naturally the facts to be seen in reproduction. It is evident that in such a difficult subject one must look for sketches, or general views, rather than theories worked out in detail. But at any rate Sachs' views are more fruitful than Nägeli's "Idioplasma," and he made a number of experimental morphological studies on their bases.

He had already arrived at the conception of the continuity of the embryonic substance before the appearance of Weismann's *Germ-plasm.* "That which has maintained itself alive, and has continually reproduced itself since the beginning of organic life upon the earth, moving steadily onward in the eternal change of all structures, in the unvarying alternation of life and death, *that* is the embryonic matter of vegetation and it is this which in certain cases differentiates itself into the two sexes in order again to unite."

He conceived of the multiplicity of plant forms as arising, on the one hand, from the phylogenetic morphological differentiation (this, however, he regarded as an "absolute mystery"), and on the other from the re-action of the common vegetable substance in response to external stimuli (automorphosis and mechanomorphosis). "Adaptation" in Darwin's sense of the expression he considered entirely superfluous, and herein he was in entire agreement with Nägeli. He expressed his views in a powerful manner in his last writings—the physiological "Notices"[1] published in "*Flora*". The manuscript found after his death, entitled "The Principles of Vegetable Formation," has been handed over to Prof. Noll for publication.

This slight sketch can give but an inadequate idea of Sachs' lifework with its abundant results as regards science; indeed I can but liken what I have written to a man striking

[1] These will shortly appear as a separate publication.

one by one a few strings of an instrument that has answered to the touch of some great musician.

One may well say with the Psalmist in speaking of his days :—

> Yet is their strength but labour and sorrow.

Nevertheless his life has borne rich fruit; his name is for ever bound up with the history of botany. He has enriched this science by the discovery of new and important facts and conceptions and by his unrivalled power of clear definition. In the nature of things it is impossible that all his theories should retain acceptance, but they have all profoundly influenced his contemporaries. There is no doubt that in any other calling Sachs would have risen to the first rank; eccentricities and narrow "specialising" were alike repugnant to him. In the last years of his life he applied himself eagerly to palaeontological and zoological studies; "I must be learning, always learning," he wrote in a letter. In spite of his incessant labours he was one of the few men of the present day who possess the gift of letter-writing and withal a spirited style, clear and trenchant.

And yet these letters, written during the last fifteen years of his life, form one long report of illness.

At last Death, who in the latter years had often drawn very near, took him gently by the hand and led him to his final rest.

K. GOEBEL.

ASSOCIATION AND DISSOCIATION.

IT is now almost universally granted that the molecular weights of volatile compounds can be directly determined by measuring their vapour densities. It is only by acceptance of Avogadro's law that chemists bring into reconciliation with the atomic theory the known facts with reference to combining proportions by weight and by volume, and physicists deduce the same law in simple manner from the kinetic theory of gases.

Until recently, direct determinations of molecular weights were only possible in the case of volatile compounds, and our knowledge of molecular weights was therefore in the main derived from the study of vapour densities. And even now this method is the only one that can be regarded as of general application. For although the researches of Raoult and the generalisation of Van't Hoff with reference to dilute solutions, lead to the view that the dissolved substance in such solutions is in a condition comparable with that of the same substance when vaporised, the consequent application of Avogadro's law to dilute solutions has not been invariably attended with success. Exceptions of a very baffling character are too frequently noticed which still await satisfactory explanation, as, for example, that the molecular weights of certain metals, determined by Ramsay from the reduction which they effect in the vapour pressure of mercury, are found to be only about half their usually accepted atomic weights. While this is the case we are therefore compelled to fall back on vapour densities as forming the final criteria in molecular weight determinations.

But as long as our knowledge of molecular weights is mainly confined to what may be learnt from the study of vapour densities it is obvious that it can only apply in all strictness to matter in the gaseous state. Of the molecules of liquids and solids we could learn little in this way, unless it were possible to show that a change

of state takes place without any change in molecular complexity, which is, in many cases at any rate, highly improbable. In fact if the vapour density of a given substance is known, it would be necessary to ascertain whether and to what extent molecular changes accompany liquefaction and solidification, before the molecular weight of the substance as liquid or as solid would be known.

A large number of attempts have been made from different sides to gain an insight into the molecular conditions of liquids and solids, and in the case of liquids the problem has now been brought within a measurable distance of a successful solution. For methods have been devised which serve to show whether the molecules of a liquid are more complex than those of its vapour, and which enable the degree of complexity to be determined approximately in certain cases.

The most successful of these methods is undoubtedly that due to Ramsay and Shields. This method is based on the fact that the molecular surface energies of different liquids are found to be the same at comparable temperatures. As the molecular surface energy is the product of the surface tension, and of the surface which contains unit number of molecules, in practice the method is reduced to the determination of the surface tension of the liquid at different temperatures, for which only simple apparatus is necessary and no special skill on the part of the experimenter is required.

The investigation of the molecular surface energy of different liquids has clearly shown that liquids must be divided into two classes. A large number of liquids turn out to have the same molecular weights as their vapours, and these liquids have therefore been termed normal or monomolecular. On the other hand certain liquids are found to have molecular weights which are greater than those of their vapours, and such liquids have been termed associating. With liquids of this last class, if M is the molecular weight deduced from the vapour density, the molecular weight of the liquid will be xM, where x is called the factor of association. As far as has been at present ascer-

tained, the hydrocarbons and their halogen derivatives, the ethers, the ethereal salts, and acid chlorides and anhydrides are monomolecular liquids. Among associating liquids one must in the first rank place those compounds which contain the hydroxyl group, as the presence of this group appears to have the greatest influence in inducing association. Thus water is found to occupy quite an exceptional position among liquids, with a factor of association that is probably higher than that of any other. The alcohols, and those acids which contain the hydroxyl group, are also associating liquids. The presence of certain other groups, among which may perhaps be reckoned the amidogen and nitro-groups, induces association, but probably in lesser degree than does the hydroxyl group.

In addition to the surface tension method, there are several other means of judging whether a liquid is monomolecular or associating. None of these are, however, so exact in principle as the method described, and the degree of association can be only roughly gauged by these processes. The chief methods are those based on an examination of the liquid when it is undergoing a change of state, either to gas or to solid, for it is fairly evident that at these points the influence of association should be rendered apparent in the most marked manner.

Several methods of this kind have been indicated by Guye, based chiefly on the behaviour of liquids either at or near their critical points. Exceptions to Trouton's law, that the molecular latent heat of vaporisation divided by the boiling-point on the absolute scale is approximately constant for all liquids, occur, as Linebarger has shown, only with associating liquids. The molecular volumes of monomolecular liquids are approximately proportional to their boiling-points on the absolute scale, the value of T/mv being about 3·5; but in the case of associating liquids this rule no longer holds and T/mv has a much higher value (in the case of water 19·85). Combining this last result with Trouton's law, it appears that the product of the latent heat of vaporisation and the density at the boiling-point (the latent heat of vaporisation of unit volume of the

liquid) is, in the case of monomolecular liquids, about 75, and has generally a higher value for associating liquids. The latent heat of fusion affords another indication of association in liquids. The latent heat of fusion of ***unit volume*** divided by the temperature of fusion on the absolute scale is approximately constant and equal to 0·1 for monomolecular liquids. Associating liquids give a higher value and one that is not far removed from $0{\cdot}1x$.

Liquids then are of two kinds, monomolecular and associating. A full recognition of this fact is of the highest importance from the physico-chemical point of view, as it serves to throw light on many of the vexed questions of physical chemistry. And in the first place it is necessary to clearly understand the character of the difference between the two classes of liquids.

Monomolecular liquids are liquids the molecular weights of which are identical with those of their vapours when in the state of perfect gas. On the liquefaction of the vapour no change takes place in molecular composition, and the liquid on being heated or cooled also suffers no change in molecular composition. Such a liquid is therefore from this point of view a perfectly stable system, the composition of which is not affected by outside influence.

Associating liquids are liquids the molecular weights of which are not identical with those of their vapours when in the state of perfect gas. In this case on the liquefaction of the vapour a change occurs in molecular composition, and the molecules of the liquid produced are more complex than those of the vapour. But the change is not complete on liquefaction, for the researches of Ramsay and Shields have clearly shown that the factor of association is not a constant in the case of any one associating liquid, and that it alters with the temperature. As the temperature falls the factor of association increases and the liquid becomes more and more complex in composition; with a rise in temperature this change is reversed. From this point of view then, an associating liquid is not a stable but a labile system, the composition of which is subject to alteration with change of temperature and perhaps other outside conditions. It is

this labile character of associating liquids which forms the most important difference between liquids of this class and those of the stable monomolecular order.

Now hitherto it has been the general custom to compare all liquids one with another in perfectly indiscriminate fashion, in the hope of establishing general relationships among liquids that might rank with those already established for gases. This method has been attended with a certain degree of success, and regularities of a striking order have been observed among the molecular volumes, the boiling-points, the molecular refractions, and other properties of liquids. But exceptions of a very puzzling order frequently arise along with the regularities observed, exceptions for which in a large number of cases it has, up to the present, been impossible to account satisfactorily. It can now hardly be doubted that one great cause of such exceptions is to be found in the fact that monomolecular and associating liquids have been indiscriminately compared one with another, when strictly speaking they are not truly comparable.

Investigation shows that such regularities as have hitherto been observed appear in most marked manner when monomolecular liquids alone are compared one with another. The exceptions arise, as might be expected, when associating liquids are included in the list, for associating liquids are neither comparable with monomolecular liquids, nor are they directly comparable with one another.

In this connection it is important to call special attention to the case of water. Water occupies almost a unique position among liquids on account of its high factor of association, which is greater than that of nearly all other associating liquids. In all comparisons of liquids one with another, water should therefore strictly speaking be given a place apart and be regarded as exceptional. Unfortunately up to the present, for practical reasons, it has been customary to select water as the typical liquid compound, and to employ it always as the standard of comparison. The choice is an unlucky one for the reasons stated, and one that should be as far as possible abandoned.

Among those properties of liquids on which association is likely to have a marked influence, and to which a peculiar interest attaches, must be ranked their behaviour as solvents. That the solvent properties of a liquid are influenced by its degree of association, is evidenced by the fact, that, as a general rule, associating liquids more readily dissolve associating liquids, and monomolecular liquids more readily dissolve monomolecular liquids, than liquids of the one class dissolve those of the other. Compounds containing hydroxyl are, generally speaking, insoluble in the hydrocarbons, in ether or in carbon bisulphide, but soluble in water and alcohol. The higher hydrocarbons, the fats and waxes, are insoluble in water and almost insoluble in alcohol, but dissolve in other hydrocarbons, in ether and in carbon bisulphide.

Not only is the behaviour of liquids as solvents influenced by their association, but in the properties of the solutions formed the influence of association can still be traced. It has been shown by Linebarger that when monomolecular liquids are mixed one with another, the densities of the mixtures are very nearly equal to those calculated from the densities of their components, no allowance being made for contraction or expansion. Nothing of the kind holds for mixtures of associating and monomolecular liquids, or for mixtures of associating liquids with one another, for here so great a contraction or expansion occurs that the law of mixtures does not apply.

Reference has been previously made to Van't Hoff's application of the gaseous laws to dilute solutions. As is well known, according to Van't Hoff, the osmotic pressure of a dissolved substance is identical with the pressure which the same substance would have in the gaseous state, if the solvent could be completely removed so as to leave the dissolved substance in that state. Solutions therefore which contain at the same temperature and in the same volumes equal numbers of dissolved molecules should have the same osmotic pressures. In the majority of cases this is found to be so, but a large number of exceptions have been observed, the osmotic pressure being

in some cases smaller and in others greater than it should be according to the above rule.

There is little difficulty in explaining those exceptions to the osmotic pressure rule which occur when the osmotic pressure found is smaller than the theoretical. Such cases usually arise when associating compounds are dissolved in monomolecular liquids, and it is now generally admitted that in these cases the dissolved compound is still in an associated condition, so that the number of molecules present in the solution is less than that calculated on the assumption that the dissolved compound is monomolecular. But if a smaller number than the calculated number of molecules is present, the observed osmotic pressure will also be smaller than that calculated, and this is what is actually found to be the case.

A greater osmotic pressure than the theoretical is met with in the case of aqueous solutions of metallic salts, and also of solutions of the same salts in formic acid and some other associating liquids. In these cases the explanation first advanced by Arrhenius is commonly accepted, and the dissolved compound is believed to be more or less in a state of electrolytic dissociation. Assuming that the molecules of the dissolved salt are dissociated into their ions, and that each ion has the same effect in establishing osmotic pressure that a separate molecule has, the observed osmotic pressure must of course be greater than that calculated on the assumption that the dissolved compound has gone into solution without any change in molecular composition.

But the abnormal osmotic pressures of electrolytes can be explained without recourse to the theory of electrolytic dissociation, and by reference solely to the facts regarding liquids which have been just considered. When a solution is formed by dissolving a given compound in a given solvent, four possible cases are presented :—

(1) The solvent and the dissolved compound may be both monomolecular.

(2) The solvent and the dissolved compound may be both associating.

(3) The solvent may be monomolecular and the dissolved compound may be associating.

(4) The solvent may be associating and the dissolved compound may be monomolecular.

In the first and second cases experience shows that the osmotic pressure of the resulting solution is usually normal, and agrees with that calculated by the Van't Hoff rule. The third case has been already considered, and it has been shown that a lower osmotic pressure than the theoretical should be, and actually is, observed. For the fourth case it is impossible at present to bring forward recognised instances, but it is highly probable that electrolytes belong to this class of solutions.

In the first place evidence is gradually accumulating that electrolytes are only formed when the solvent is associating. Solvents which, with dissolved metallic salts, yield solutions having a high electrical conductivity, usually contain the hydroxyl group, and are associating. And other associating solvents which do not contain the hydroxyl group, as, for example, acetone, propionitrile and nitroethane, have been lately shown by Miss Aston and P. Dutoit to yield with metallic salts solutions of high conductivity. On the other hand, Kablukoff found that when a monomolecular solvent is taken, the resulting solution has practically no conductivity. That the dissolved salt is either monomolecular or approaches to this condition cannot be shown directly, as no satisfactory examination of metallic salts in the liquid state can be carried out. But ethereal salts as a class are monomolecular compounds, and analogy would therefore point to this being true of the metallic salts. It will also be noticed that those compounds which dissolve in water and other associating solvents without formation of electrolytes belong to the class of associating compounds; as, for example, alcohol, glycerol, and cane sugar.

Now it has been pointed out that when, as in the instances last quoted, the solvent and the dissolved substance are both associating (Case II.) normal osmotic pressures are obtained, although when the solvent is mono-

molecular and the dissolved substance is associating (Case III.) lower values than the normal are obtained. This is not what one would at first expect, for there seems no immediate reason why, in an associating solvent, a dissolved substance which is also associating should give values which are different to those obtained when the solvent is monomolecular. It is evident in fact that the difference in the solvent exercises the determining influence in these cases and not the character of the dissolved substance, as otherwise an associating compound would in *all* solvents give lower values for the osmotic pressure than the normal. The difficulty has been usually explained by assuming that an associating solvent brings about a dissociation in any dissolved compound of an associating type, and reduces it to the monomolecular condition. The dissociation here spoken of is not electrolytic dissociation, but a breaking down of complex molecules into simple ones.

It is difficult to admit the universal dissociating influence thus assigned to associating solvents, without any explanation of the why and wherefore of its existence. For according to this view every compound, whatever its character, when dissolved in any associating solvent, must be regarded as undergoing dissociation. If the compound is associating, the action merely proceeds as far as the breaking up of the complex molecules into simple ones, and the dissociation is therefore of the ordinary kind. But if the compound is monomolecular, the action is of a more complex character, for the molecules themselves are broken up, and so-called electrolytic dissociation occurs.

A far simpler view of the matter is obtained if it is allowed that the abnormal behaviour of solutions containing an associating solvent is not due to any change in the dissolved compound, but due solely to the character of the solvent itself, and its influence on the osmotic pressure. It has been already pointed out that monomolecular and associating liquids are not directly comparable one with another. Admitting the validity of the osmotic pressure formulæ when the solvent is a monomolecular one, it does not therefore follow that these formulæ will necessarily

apply when the solvent is associating. The probability is that some suitable modification is required and the introduction of a factor due to the associating character of the solvent.

It can be shown that, if the influence which the association of the solvent is here supposed to exercise on the osmotic pressure be admitted, the osmotic pressure of a solution containing an associating solvent should always be greater than that of a solution of similar concentration with a monomolecular solvent. Solutions of Class II. might therefore be expected to exhibit osmotic pressures that would approximate to the normal calculated values, and solutions of Class IV. would have osmotic pressures greater than the normal. It fact solutions of Class IV. would possess the behaviour met with among electrolytes, and as shown above electrolytes do in all probability belong to this class of solutions.

The supporters of the theory of electrolytic dissociation deny that the character of the solvent can have any direct influence on the osmotic pressure of a solution. The thermo-dynamical treatment of the subject leads to this conclusion, and any contrary view argues a flaw in the train of reasoning by means of which this result has been obtained. The difficulties that arise in applying thermo-dynamics to chemical investigations have been well illustrated by Fitzgerald in his Helmholtz Memorial Lecture. And a difficulty which has been overlooked occurs in dealing with dilute solutions. A monomolecular solvent does not change in composition with rise or fall of temperature, but any change in temperature at once alters the composition of an associating solvent. As temperature enters as one of the factors into thermo-dynamical equations, in the treatment of dilute solutions some distinction should be drawn between those which contain monomolecular and those which contain associating solvents. But up to the present no such distinction has been made and all liquids have been treated as exactly alike.

In opposing to the theory of electrolytic dissociation the view that association of the solvent influences the osmotic

pressure of solutions, and that the exceptional behaviour of electrolytes is due to this cause, it must be urged that the results of the thermodynamical treatment of this question have not up to the present been conclusive. A full recognition is necessary of the fact that in accordance with their differences in composition it is necessary to sharply divide liquids into two different classes. The treatment applied to liquids of the one class cannot be indiscriminately extended to those of the other, for if this is done the results obtained will be of an altogether misleading character.

HOLLAND CROMPTON.

RECENT EXPERIMENTS IN HYBRIDISATION.

IT has long been recognised that the facts of hybridisation have an important bearing on many subjects of biological interest. The question of the infertility of crosses was very carefully investigated by Darwin, and since his time has been more than once discussed afresh; while the structural and other characters presented by hybrid offspring are of undoubted weight in reference to the problem of heredity.

For some years past Dr. M. Standfuss, of Zürich, has been experimenting on a large scale in the production of insect hybrids. The results of these experiments, some of which confirm previous views, while others appear to suggest conclusions not hitherto reached, have now been collected by their author in his *Handbuch der paläarktischen Gross-Schmetterlinge*, published at Jena in 1896. Many of the data thus made available derive especial value from the fact that they are expressed numerically, and the whole series forms a noteworthy contribution to our knowledge of the subject. It is proposed to give here a short account of these experiments, with their results, for the benefit of those readers to whom the original records may be difficult of access.[1]

The author prefaces his account with a list of fertile and infertile pairings between different species of macrolepidoptera that have been observed by himself and others, both under natural conditions and in captivity. Details are first given of twenty-four cases of pairing between *Bombyx neustria* L. ♂ and *B franconica* Esp. ♀. The results showed every transition between complete absence of issue and the deposition of eggs normal in numbers and fertility. Failure was in some instances plainly due to inadaptability of the genital apparatus. On this series of pairings Stand-

[1] Several of Dr. Standfuss's specimens have lately been exhibited in London, at the rooms of the Royal Society, Burlington House and at the Natural History Museum, South Kensington.

fuss remarks that the absence of uniformity in the results shows that several trials should be made before any given cross is pronounced infertile.

Five crosses are mentioned from which only male offspring are known to have resulted; these are *Deilephila porcellus* L. ♂ and *D. elpenor* L. ♀; *Smerinthus austauti* Stgr. ♂ and *S. atlanticus* L. ♀; *Fumea nitidella* Hof. ♂ and *F. affinis* Rutt. ♀, with the reciprocal cross *F. affinis* ♂ and *F. nitidella* ♀; *Bombyx neustria* L. ♂ and *B. franconica* Esp. ♀.

In the following five cases only female offspring have been observed, and these contained no eggs capable of development: *Bombyx neustria* L. ♂ and *B. castrensis* var. *veneta* Stdfs. ♀; *B. franconica* Esp. ♂ and *B. castrensis* var. *veneta* ♀; *B. quercus* L. ♂ and *B. trifolii* Esp. ♀; *Saturnia pyri* Schiff ♂ and *S. pavonia* L. ♀; *Drepana curvatula* Bkh. ♂ and *D. falcataria* L. ♀.

In the following seven the males predominate and the females are again sterile: *Deilephila euphorbiæ* L. ♂ and *D. vespertilio* Esp. ♀; *D. hippophaes* Esp. ♂ and *D. vespertilio* Esp. ♀; *Smerinthus ocellatus* L. ♂ and *S. populi* L. ♀; *Saturnia spini* Schiff ♂ and *S. pyri* Schiff ♀; *S. spini* Schiff ♂ and *S. pavonia* L. ♀; *Harpyia vinula* L. ♂ and *H. erminea* Esp. ♀; *Notodonta dromedaria* L. ♂ and *N. torva* Hb. ♀.

The author points out that no fertile female offspring having been observed in any of the above seventeen crosses, none of these hybrids, so far as our present knowledge goes, could continue their race. It is unfortunate that the actual numbers of the broods in question are not given; in some instances, as in that of *B. neustria* ♂ and *B. franconica* ♀ they are presumably large.

In three more crossings the offspring consisted of both sexes in normal proportions; the females, however, contained at best but few eggs, and these abnormal in structure. The crossings were of *Smerinthus populi* L. ♂ and S. *ocellatus* L. ♀; *Saturnia pavonia* L. ♂ and *S. pyri* Schiff ♀; *S. pavonia* L. ♂ and *S. spini* Schiff ♀.

Finally, in two more the sex-distribution appears again

to be as usual, and the females contain ova normal in appearance. Their fertility, however, has not been proved, and Standfuss thinks he has reason to doubt it. These are *Zygæna trifolii* Esp. ♂ and *Z. filipendulæ* L. ♀ ; *Biston hirtarius* Cl. ♂ and *B. pomonarius* Hb. ♀.

In no single instance, according to Standfuss, has the female of any true hybrid among lepidoptera been shown experimentally to be fertile. Haeckel's counterstatement with regard to the genera *Saturnia* and *Zygæna* is probably erroneous. [1]

The most complete series of Standfuss's experiments in hybridisation is one carried on for over ten years with three species of *Saturnia*, *viz.*, *S. pavonia* L., *S. spini* Schiff, and *S. pyri* Schiff. The results of these experiments are recorded and compared by their author with great minuteness, and excellent figures are given by him of several of the resulting forms in their immature and final stages. A brief outline of these records, omitting details, will here be attempted.

1. *Saturnia pavonia* ♂ and *S. spini* ♀. In this hybrid, called by Standfuss *S. bornemanni*, the eggs first laid were regularly deposited, and were fertile to the extent of from 60 to 80 per cent. The larvæ in all five stages bore a much closer resemblance to those of *S. spini* than to those of *S. pavonia*, though this became less pronounced after the second stage was passed. The cocoon and pupa were both intermediate in structure, but the perfect insect was nearer to *S. spini* than to *S. pavonia*. This applies to both sexes, but is more easily seen in the male, the males of the two parent species differing (as is usual) more than the females.

2. The reciprocal cross (*S. spini* ♂ and *S. pavonia* ♀ ; = *S. hybrida* Ochs.) has not been produced in captivity,

[1] Standfuss does not notice the case cited from Quatrefages by Wallace (*Darwinism*, 1889, p. 163), of fertility *inter se* in the hybrids between *Bombyx cynthia* and *B. arrindia*. Fletcher also has obtained fertile hybrids of both sexes between *Zygæna loniceræ* Esp. and *Z. trifolii* (*Proc. Ent. Soc. Lond.*, 1893, p. ix.). On the other hand, the hybrids of *Z. loniceræ* and *Z. filipendulæ* proved infertile (*Ibid.*, 1891, p. ix.), and the hybrid progeny of various species of *Platysamia* and *Actias* were found by Miss Morton to be sterile in both sexes (*Ibid.*, 1895, p. xxxiv.).

but is repeatedly found in the wild state, generally as a larva. The question naturally suggests itself, how the parentage of this hybrid is known. Standfuss replies that the larva, though intermediate in character, shows constant differences from that of *S. bornemanni*, and that the conditions in nature for the pairing of *S. spini* ♂ and *S. pavonia* ♀ are favourable, whereas those for the converse cross are difficult or impossible. The males of both species emerge before the females, and in regions where both occur *S. pavonia* is out first. Hence, though the females of *S. pavonia* are out with the males of *S. spini*, the converse does not take place. It must be allowed that it would be well to test this conclusion as to the parentage of *S. hybrida* by experiment. The larva of *S. hybrida* resembles *S. pavonia* somewhat more than does that of *S. bornemanni;* it possesses, however, the greasy polish of *S. spini* to a greater extent than the latter. The cocoon and pupa both show a nearer approach to *S. spini* than do those of *S. bornemanni*, and the same applies to the perfect insect; in fact the resemblance of both sexes to the male parent is remarkably close.

3. *S. pavonia* ♂ and *S. pyri* ♀. This crossing produced hybrids which fell into two classes, a dark form called by Standfuss *S. daubii*, and a paler form to which he gives the name *S. emiliæ*. The latter is by far the commoner. As in former cases, if the laying is much deferred after pairing, the eggs are apt to prove infertile. The larva in its early stages is very like that of *S. pyri;* it becomes more and more like that of *S. pavonia*, and finally bears a close resemblance to the latter species. The cocoon is intermediate; the pupa is nearer to *S. pavonia* than to *S. pyri*. The perfect insect, except for a reduction in the sexual disparity of size, is more like an enlarged *S. pavonia* than a diminished *S. pyri*. In a majority of specimens some of the veins were forked terminally at a greater or less distance from the margin of the wings.

The males of this hybrid paired readily with the females of *S. pavonia;* they were also attracted by the female hybrids, though in a less degree. The latter were, as already

stated, infertile, the oviducts containing no ripened eggs. Pairing also took place, though with much less readiness than in either of the preceding cases, between the hybrid males and some females of *S. pyri.* The number of fertile eggs produced in the latter case was far smaller than in the cross with *S. pavonia.*

4. *S. bornemanni* ♂ and *S. pavonia* ♀ (*S. bornemanni* is the cross-product of *S. pavonia* ♂ and *S. spini* ♀). The larvæ at first closely resembled those of *S. pavonia*, but in subsequent stages the influence of *S. spini* began to assert itself. They presented a very variable appearance on reaching the fourth stage, which period unfortunately they did not survive. Fresh batches, however, have since been raised to maturity, though no record of their history has yet been published.[1]

5. *S. emiliæ* ♂ and *S. pavonia* ♀ (*S. emiliæ* is a cross-product of *S. pavonia* ♂ and *S. pyri* ♀). The larvæ of this hybrid (named *S. standfussi* by Wiskott) are again very variable. Their general appearance is that of a large *S. pavonia.* The cocoon and pupa are also near the same parent species. The perfect insect, like the larva, is variable; it always, however, shows much resemblance to *S. pavonia*, and the sexes are dissimilar as in that species. The margins of the wings are apt to be scalloped. The oviducts of the only female that emerged contained mature eggs, but only about twenty, or one-tenth of the normal number in *S. pavonia* or *S. pyri.* It is possible that these hybrids may be fertile *inter se.*

6. *S. emiliæ* ♂ and *S. pyri* ♀ (*S. risii* Stdfs.). This pairing was only obtained with great difficulty, and in four cases out of nine the eggs, though laid in normal numbers, did not hatch. In the remaining five broods only one per cent. produced caterpillars. These at first closely resembled *S. pyri*, and in the second stage still showed more likeness to *S. pyri* than to the male parent form. In the third stage the *S. pyri* characters began to be lost, and in the fourth

[1] Specimens of this hybrid have been reared by the present writer from eggs kindly sent him by Dr. Standfuss in 1895. Other individuals raised from the same batch of eggs are in the Hope Collection at Oxford.

those of *S. pyri* and *S. pavonia* were fairly balanced. After changing its last skin the larva resembles a large *S. pavonia*, though its parentage is three parts *S. pyri*. The perfect insects, of which only six were reared, were in many respects remarkable. Unlike *S. pyri* they were sexually dimorphic, the females differing little from those of *S. emiliæ*, while the males showed a nearer approach to *S. pyri*. The females were not dissected, but were probably sterile. Of the six specimens, one was hermaphrodite and three others showed a tendency in that direction. These four were the produce of three separate females out of the five that laid living ova. Standfuss draws attention to the fact that the normal occurrence of hermaphrodites in lepidoptera is given by Speyer as about 1 in 30,000. The latter number he considers rather too low than too high. There was no hermaphroditism in any known member of the families of the progenitors of these hybrids; and its appearance in this proportion must be, he thinks, a consequence of their exceptional origin. On the other hand, he has bred more than 1000 genuine hybrids without one such case occurring,[1] nor was any such tendency shown by a single one of the sixteen specimens of *S. standfussi*—a form whose origin is analogous to that of the present cross-product.

In the well-marked hermaphrodite mentioned above, the distribution of sexual characters is remarkable. The shape of the fore wings is rather female than male, the colouring on the upper side of both is male; on the under side, the right is mostly male and the left female. In both hind wings the upper surface has the costal portion male, the remainder female; the right, which is about one-fifth larger than the left, has the female area more extensive. The under surface of the right or larger hind wing is female, of the left mostly male. The right antenna is male in form; the left partly male and partly female. The external genital organs on the right side are of a malformed male type; on the left side absent.

[1] Barrett (*Lepidoptera of the British Islands*, vol., ii., 1895, pp. 10, 11) gives some details of the cross between *Smerinthus ocellatus* and *S. populi*. These are said to be "often gynandrous".

The want of vigour in this cross is shown by the fact that out of nine pairings, each resulting in an average of 200 eggs, only ten larvæ were produced; and of these only six, as we have seen, attained the perfect condition.

7. *S. pavonia* ♂ and *Actias luna* L. ♀. Nine apparently normal pairings took place, and over 1000 eggs were laid; but none of these hatched.

8. *S. pavonia* ♂ and *A. isabellæ* Graëlls ♀. As a result of this crossing, ninety-eight eggs were laid, seven of which hatched. The caterpillars however did not long survive their first change of skin.

9. *S. bornemanni* ♂ and *S. pyri* ♀. As *S. bornemanni* is the cross-product of *S. pavonia* ♂ and *S. spini* ♀, this hybrid is descended from all three species.[1] Only one pairing was obtained, and 92 per cent. of the eggs hatched. Some of the resulting perfect insects were lately shown in London with the other exhibits of Dr. Standfuss, who promises to publish further details of their history.

Relative phylogenetic age of the three European species of Saturnia. Before stating the general conclusions at which he has arrived as a result of the above series of experiments, the author proceeds to discuss the question of the relative phylogenetic age of the three species *Saturnia spini*, *S. pavonia* and *S. pyri*. Of these he considers *S. spini* to be the oldest form and *S. pyri* the most recent. His main reasons are briefly as follows:—

(1) The larva of *S. spini* maintains its original black colour throughout its life. *S. pavonia* loses this sometimes in the third stage, always in the fourth and fifth. *S. pyri* abandons it almost completely in the third stage and onwards. The succeeding green colour, which is no doubt adaptive, is acquired by *S. pyri* at an earlier stage and more completely than by *S. pavonia*.

(2) In the larva of *S. spini* the tubercles are not very prominent even in the adult, and the knobs at their summit are not distinctively coloured until the last stage. In *S.*

[1] Mr. A. G. Butler informs me that an analogous triple cross has been obtained between a goldfinch and the hybrid issue of the English and Japanese greenfinch.

pavonia the tubercles are conspicuous and the knobs acquire an appearance distinct from the general surface of the body in the third or fourth stage. In *S. pyri* on emergence from the egg the knobs are already coloured and indications exist of the extreme prominence of the tubercles.

(3) The tactile bristles are least developed in *S. spini*, most in *S. pyri.*

(4) The cocoon of *S. spini* is simpler than that of *S. pavonia.* That of *S. pyri* is the best defended of the three.

(5) *S. spini* is almost sexually monomorphic, though the male is somewhat the smaller. The female is very sluggish, and the antennæ of the male are pectinate to a high degree. In *S. pavonia* there is well-marked sexual dimorphism, as regards both size and colouring. The female resembles that of *S. spini* in aspect ; it is less sluggish, though not very active. The antennæ of the male are less strongly pectinate. *S. pyri* again is sexually monomorphic ; the female is a tolerably good flier, and the antennæ in the male are less strongly pectinate than in many other species of *Saturnia.*

Judging from the perfect insects alone, we must conclude, according to Standfuss, that the less specialised *S. spini* is older than *S. pavonia.* We should, however, find it difficult to determine whether *S. pyri* arose before or after the divergence of these two. The larval and pupal stages enable us to answer the question. The three species form a progressive series in protective adaptation, *S. spini* always taking the lowest and *S. pyri* the highest step in the scale. It must therefore be concluded, on the whole evidence, that *S. spini* is phylogenetically the oldest, and *S. pyri* the youngest of the three forms.

General conclusions with reference to hybridisation between distinct species. As the author elsewhere expresses it, the crossing of two distinct species gives rise to a "Zwischenform" but not to a "Mittelform". The Mittelform may, however, exist as a temporary stage in larval growth. This depends on the following principles, which Standfuss considers to be warranted by the above experiments with species of *Saturnia* :—

1. The freshly-hatched larva closely resembles the female parent.

2. With the process of growth a resemblance to the male parent gradually increases.

3. The final extent of approximation towards the male parent depends on the relative phylogenetic age of the two species; the older being able to transmit its properties, whether of structure or habit, better than the younger.

Thus the crossing of *S. pavonia* ♂ with the phylogenetically younger *S. pyri* ♀ gives rise to a larva in which at first the maternal and afterwards the paternal characters predominate.[1] The resulting perfect insect is by more than two-thirds of its external appearance *S. pavonia*, and by less than one-third *S. pyri*. Its habits and functions correspond with its external aspect. It prefers to fly by day, like *S. pavonia* ♂, and pairs easily with the female of that species, from 43 to 62 per cent. of the eggs being fertile. On the other hand, it does not pair readily with *S. pyri*, and the resulting eggs on an average of nine cases gave only one larva from 180.

Similarly *S. pavonia* ♂, when paired with the phylogenetically older *S. spini* ♀, gives a form of which in the perfect state about two-thirds of the external aspect belong to the type of *S. spini*. The male flies by night. After crossing with *S. pavonia* ♀ the resulting eggs were only fertile to the extent of 16 to 22 per cent.; while the crossing with *S. spini* ♀, though not easily brought about in consequence of their diverse times of appearance, yielded eggs of which from 94 to 98 per cent. were fertile.

Thus the male *S. pavonia* is able to influence the issue of the relatively gigantic *S. pyri* ♀ much more than that of *S. spini* ♀.

Again, the issue of *S. spini* ♂ and *S. pavonia* ♀ is much nearer *S. spini* than is that of *S. pavonia* ♂ and *S. spini* ♀. Hence,

4. In reciprocal pairing the male is able to transmit the characters of the species in a higher degree than the female.

[1] Barrett (*loc. cit.*, p. 11) says, quoting Porritt, that the larvæ of the cross between *Smerinthus ocellatus* and *S. populi*, "though like those of *S. populi* when young, appear to become intermediate, or even to resemble those of *S. ocellatus* when full grown".

The above cases show that the former influence, *viz.*, that of the older-established species, is the more effective of the two. The sexual prepotency of the male *S. pavonia* counts for less than the specific prepotency of the female *S. spini*. The highest effect of course is produced when the two influences concur, as in the hybrid of *S. spini* ♂ and *S. pavonia* ♀.

A further result of the experiments is that while no female hybrid was proved to be fertile, there are undoubted cases of fertility in male hybrids. This has been shown by crossing with the females of both parent species, and in one case with a female of a third species (*S. bornemanni* ♂ and *S. pyri* ♀).

Hence hybridisation is not necessarily a merely transitory phenomenon. There seems to be no reason why a male hybrid should not propagate by "back-crossing" under natural conditions; and since the product of this kind of crossing is not found to show a complete reversion to the type of the female parent, it is possible that the existence of various intermediate forms in such genera as *Melitæa*, *Zygæna* and *Agrotis* may be accounted for in this manner. Cases of simple pairing between distinct species of the two former genera have been observed by the author in nature.

The above conclusions with the facts on which they rest, though much condensed from the original, have been stated as far as possible in Dr. Standfuss's own words. A few brief comments seem called for.

(1) The rule laid down by Standfuss as to the prepotency of the phylogenetically older species is probably another expression of the fact so clearly established by Darwin[1] that hybridisation frequently leads to reversion. It is significant that Standfuss considers the hybrid form *S. emiliæ* as partly reproducing an ancestral stage in the history of *S. pavonia* rather than the form of that species at present existing.

(2) It is well known that when plant hybrids are crossed with one of the parent species, considerable variability may

[1] *Animals and Plants under Domestication*, 1868, vol. ii., p. 254, etc.

occur in the offspring (Focke, quoted by Weismann). The facts above recorded as to the results of back-crossing (see accounts of *S. standfussi*, *S. risii*, etc.), show that the same rule holds good with hybrid *Saturnias*.

(3) Some of the above generalisations, at present resting on only a few instances, can hardly be regarded as more than provisional. It will be interesting to see whether the further results of these experiments, which are being continued by their author, tend to confirm or to modify the conclusions previously reached.

Cross-breeding between local races. Akin to the above experiments are others in which local races of the same species were paired. These gave results in many respects analogous with the foregoing.

1. *Callimorpha dominula* L. ♂ and *do.* var. *persona* Hb. ♀. The crossing of *C. dominula* ♂ with var. *persona* ♀, the latter being a local race found only in Tuscany and Calabria, generally produced issue that, though very variable in the perfect state, bore on the whole a closer resemblance to *C. dominula* than to var. *persona*. In one case, however, a majority of the brood came nearer to the latter, and one individual even went beyond the *persona* type. From 3 to 5 per cent. of the eggs were sterile.

2. *C. dominula* var. *persona* ♂ and *C. dominula* ♀. In this, the reciprocal cross, the resulting perfect insects again varied between the parental types, but, on the whole, came nearer to *C. dominula* than to the variety, though less so than those of the former cross. Of the eggs, from 10 to 15 per cent. were sterile, from which Standfuss concludes that the male of var. *persona* has already diverged from the physiological standard of the species.

The products of each crossing are fertile in both sexes, but Standfuss is unable to say whether they have undergone any diminution in fertility as compared with the parent forms.

3. *Ocnogyna hemigena* Grasl. ♂ and *O. zoraida* Grasl. ♀. These are regarded by the author as local races of the same species, the former inhabiting the Pyrenees and the latter the mountains of Andalusia. The cross-product (called by

Staudinger *O. zoragena*) resembles a large *O. hemigena.* According to Kröning the mongrel issue are fertile *inter se*, but quickly degenerate in size and vitality. This of course may be due to other causes than their mixed origin.

4. *Spilosoma mendica* Cl. ♂ and *do.* var. *rustica* Hb. ♀. The larvæ were nearly always formed within the egg; but in some broods not one, and in others only from 8 to 12 per cent. hatched. In one case, however, as many as 93 per cent. of the eggs gave living larvæ. All the broods suffered severely from disease. The perfect insects, which did not show much variation, diverged only slightly in appearance from var. *rustica.* The males were mostly light-coloured, as in that variety, and the darkest of them were far lighter than any male of *S. mendica.*

5. *S. mendica* var. *rustica* ♂ and *S. mendica* ♀. The reciprocal cross, which failed with Standfuss in consequence of an epidemic among the larvæ, was obtained by Caradja. Pupæ sent by him to Standfuss gave thirty-one perfect insects, seventeen being males and fourteen females. These were more variable than the former cross-product, but on the whole inclined towards var. *rustica.*[1]

It is observable that in this case of reciprocal crossing, the influence of the females appears to be equal to if not greater than that of the males. This shows that the generalisation as to male prepotency founded on the case of the *Saturnias* is not of universal application. It will be remembered also that the issue of one of the pairings between *C. dominula* ♂ and var. *persona* ♀ showed female prepotency, though the general result in the case of this and the reciprocal cross tended in the opposite direction.[2]

Standfuss considers that the present experiments with species and their local races favour his view as to the superior influence of the phylogenetically older form.

Aberrations. Some very remarkable facts are recorded

[1] This corresponds, as has been pointed out by Mr. South, with the result obtained by Mr. Adkin, who effected the same cross in 1889. *Vid. Entomologist*, for August, 1897, p. 206; *Proc. Ent. Soc. Lond.*, 1890, p. xl.

[2] See also p. 199, *infra*, note.

as to the effect of crossing a sport or aberration with its parent form. The result, which is entirely different from that which follows the crossing of distinct species, or even of local races, may be broadly stated as follows: When an aberration is crossed with its parent form the issue is sharply divided, in both sexes, into specimens of the aberration and of the normal form of the species.

Thus in the dark aberration *zatima* Cr. of *Spilosoma lubricipeda* Esp., there are many degrees from the least dark form of the aberration (ab. *intermedia* Bang-Haas) up to the darkest (ab. *deschangei* Depuis); but no transitional forms occur to bridge over the wide gap between *intermedia* and *lubricipeda*, nor can they be produced by crossing these two. "It seems," so Standfuss expresses it, "as if there were antagonistic characters which cannot coexist in the same individual".[1] Instances follow which will be briefly noticed here. For full details, which are of great interest, the reader is referred to the *Handbuch*, pp. 305-321.

1. *Spilosoma lubricipeda* Esp. ♂ and *do.* var. *zatima* Cr. ♀. These, crossed by Burckhardt in 1889, gave *lubricipeda*, *intermedia* and *zatima* (*intermedia* being, as just stated, merely a less dark form of *zatima*). Two of these *intermedia* were paired, giving again *lubricipeda*, *intermedia* and *zatima*. In this third generation several pairings were effected, as follows: *zatima* ♂ and *lubricipeda* ♀; *lubricipeda* ♂ and *zatima* ♀; *intermedia* ♂ and *do.* ♀; *intermedia* ♂ and *zatima* ♀. All these gave *lubricipeda*, *intermedia* and *zatima* in varying proportions, except the cross *lubricipeda* ♂ and *zatima* ♀, from which only *zatima* resulted. A pair of *lubricipeda* from this fourth generation gave a brood of 34 *lubricipeda* and 1 extreme *zatima*.

In all these successive broods, carried on into the fourth year from the date of the original pairing, there were no transitional forms between *lubricipeda* and *intermedia*.[2]

[1] The bearing of this may be noted on the theory of "determinants". See Weismann, *Germ-plasm*, 1893, pp. 293, etc. See also Bateson, *Materials for the Study of Variation*, 1894, esp. Intro., Sect. iii.; and this Review, 1897, pp. 554-569.

[2] The above case of cross-breeding between colour-varieties of the same race, in which there are no intermediates and the colours of the ancestors

2. *Psilura monacha* L. ♂ and *do.* ab. *eremita* O. ♀. In 1893 Standfuss raised a brood from a pair of normal *P. monacha* received from Silesia. This brood contained one female specimen of the dark aberration *eremita*, which was paired with a normal *P. monacha* ♂ from Zürich. The issue consisted of 22 (2 ♂ and 20 ♀) typical *monacha*, 23 (18 ♂ and 5 ♀) typical *eremita*, and 6 forms (5 ♂ and 1 ♀) in which the characters of the two are unsymmetrically mixed, not harmoniously blended. There is no apparent tendency to hermaphroditism. This unsymmetrical mixture resembles that occasionally seen in the male of *Ocneria dispar* L., where also, according to Standfuss, the patches of the female pattern that sometimes appear do not betoken organic hermaphroditism.

3. *P. eremita* ♂ and *P. monacha* ♀. This was a natural pairing found by Standfuss in Silesia. The result was entirely different from that of the former cross, inasmuch as the issue contained every kind of transition between the two parent forms, while a few even transcended the male parent in darkness.

Thus the two specimens of so-called *eremita* in this and the former observation, though externally so much alike, possessed entirely different properties in regard to the power of transmission to descendants. Standfuss explains the apparent contradiction thus: The first *eremita* was a true sport or aberration, and in its case the rule held good as usual. The second, also called "*eremita*" (which did not show the *eremita* characters so well as some of its own offspring), was a link in the chain leading by slight variations to a darker and presumably better protected form of *P. monacha*, which, under the influence of natural selection, is gradually developing itself in certain parts of the range

of 195 individuals are known for three generations, seems, like that of *Aglia tau* and its aberration *lugens* (*infra*, p. 199), to promise suitable data for the verification of Mr. Francis Galton's law of heredity. According to this law the sum of the ancestral contributions is expressed by the series $(0{\cdot}5) + (0{\cdot}5)^2 + (0{\cdot}5)^3 + \ldots (0{\cdot}5)^n$, each term standing for a generation. See Galton, *Natural Inheritance*, 1889, p. 134, and "Average Contribution of each Several Ancestor to the Total Heritage of the Offspring," *Proc., Roy. Soc.*, 1897, pp. 401-413.

of the species. It took rank therefore not as an aberration, but rather as a member of a local race, and with this its behaviour accorded.[1]

4. *Aglia tau* L. ♂ and *do.* ab. *lugens* Stdfs. Like the dark aberration *zatima* of *S. lubricipeda*, the dark *Aglia tau* (called by Standfuss ab. *lugens*) exists in several degrees of development, from the "ab. *fere nigra*" of Thierry-Mieg to the "ab. *nigerrima*" of Bang-Haas. All these different stages of darkening have been obtained by Standfuss from pairings between *A. tau* and ab. *lugens*, but no transitional form between the normal *A. tau* and ab. *fere nigra* has ever been so produced.

In 1888 Standfuss crossed males of ab. *lugens*, which had been interbred for two generations, with females of the normal *A. tau*. In 1889 *lugens* derived from these were employed in the following pairings: *lugens* ♂ and *tau* ♀; *tau* ♂ and *lugens* ♀; *lugens* ♂ and *do.* ♀. The specimens of *A. tau* were in each case of different ancestry from the *lugens* stock. In 1890 two separate pairings were procured of *lugens* ♂ and ♀ from the third of the above broods, so that the perfect insects emerging in 1891 had both parents and all grandparents of the *lugens* type. The results of these experiments were curious, and should be studied in the original account (*Handbuch*, p. 312). Here it may be briefly stated that of the 1889 pairings, the first two (*lugens* ♂ and *tau* ♀; *tau* ♂ and *lugens* ♀) produced about 50 per cent. of each form.[2]

The third (*lugens* ♂ and ♀) gave 36 per cent. of *tau* to

[1] Compare the result of crossing *C. dominula* with the local race *persona*, and see especially Standfuss's figures, *Op. cit.*, Taf. V. Compare also the case of *A. betularia* and its aberration *doubledayaria* (*inf.*, p. 201), which is strikingly analogous with that of *P. monacha*.

[2] In both of these reciprocal crosses there is a slight preponderance of forms resembling the *male* parent—in the first instance *lugens*, in the second *tau*. This case is fairly analogous with that of the breed of Basset hounds lately investigated by Mr. Francis Galton ("The Average Contribution of each Several Ancestor," etc., *Proc. Roy. Soc.*, 1897, pp. 401-413), where the reciprocal crosses of two colour-varieties show a male prepotency in the proportion of about six to five (*loc. cit.*, Table I., p. 409). As in the instance of *tau* and *lugens*, there are no intermediate forms between

64 per cent. of *lugens*. In the 1890 broods, all of whose ancestors were *lugens* for two generations, the proportion of *tau* had fallen to a little over 11 per cent. in one and a little under the same figure in the other. In each of the five cases about twice as many females as males were of the *tau* form. Hence, as Standfuss puts it, it is more difficult to transform the female of *Aglia tau* than the male.[1]

5. *Grammesia trigrammica* Hufn. ♂ and *do.* ab. *bilinea* Hb. ♀. The female *bilinea*, taken by Gross at Garsten in Austria, laid eggs of which the male parent was presumably a normal *G. trigrammica*. Of the sixty-seven perfect insects that resulted, thirty-eight were *trigrammica* and twenty-nine *bilinea*. There were no intermediates.

6. *Angerona prunaria* L. ♂. and *do.* ab. *sordiata* Fuessl. ♀. This cross, procured by Zeller, gave seventeen *prunaria* and fourteen *sordiata*.

7. *A. prunaria* ab. *sordiata* ♂ and *A. prunaria* ♀. This cross, also obtained by Zeller, gave eighty-four of *prunaria* to sixty-eight of *sordiata*, *i.e.*, as in the reciprocal cross, about 55 per cent. of the type and 45 per cent. of the aberration. In neither of these cases were there any intermediates.

8. *A. prunaria* ab. *sordiata* ♂ and ♀. Among a large brood reared from the eggs of a pair of normal *A. prunaria*, there appeared three males and two females of the aberration *sordiata*. From a pair of these Standfuss obtained thirteen *prunaria* (three ♂ and ten ♀) and forty-two *sordiata* (twenty-four ♂ and eighteen ♀). Again there were no transitional forms.

9. *Amphidasis betularia* L. ♂ and *do.* ab. *doubledayaria* Mill. ♀. A female *doubledayaria* found by Steinert near

the "tricolour" and "non-tricolour" hounds, though there are different degrees of development of black in the "tricolours" which may be comparable with the range of variation between "*fere nigra*" and "*nigerrima*" in *lugens*. It is noticeable that in the case of *S. lubricipeda* and *do.* ab. *zatima* (*supr.* p. 197), the reciprocal crosses do not afford evidence of male prepotency. The numbers, however, are small.

[1] Compare the result of the cross between *P. monacha* ♂ and ab. *eremita* ♀. Here the typical *monacha* form was retained by twenty females and only two males.

Dresden produced seventy-five *betularia* (thirty ♂ and forty-five ♀) and ninety *doubledayaria* (thirty-four ♂ and fifty-six ♀). The male parent was doubtless an ordinary *betularia*. Two of the examples classed as *betularia* were darker than the normal, but otherwise no transitional forms occurred. Standfuss is of opinion that even these two need not be regarded as owing their darker coloration to the cross, for it is well known that *A. betularia*, like *P. monacha*, is undergoing a gradually increasing melanism, which is probably protective, in many parts of its area of distribution. The extreme aberration *doubledayaria*, which thirty years ago was known only from Great Britain, has now appeared in Westphalia, the Rhine Provinces, Hanover, Gotha, and lastly in Dresden and Silesia. In several of these places it is becoming more and more common, and in at least some of them it is found side by side with the darkening forms of *A. betularia*, which, though of different nature and origin from the sport *doubledayaria*, are no doubt being preserved and brought up to its level (in aspect) under the influence of natural selection.[1]

10. *Boarmia repandata* L. ♂ and *do.* ab. *conversaria* Hb. ♀. A large brood raised from the eggs of a pair of normal *B. repandata* contained three males and one female of the aberration *conversaria*. This female, which was paired with a wild male *B. repandata*, produced twenty-eight *repandata* (of which ten were males and eighteen females) and six *conversaria* (four being males and two females). The majority of the larvæ died during the winter. Here again intermediate forms were entirely absent.[2]

From the above experiments in the pairing of normal forms with aberrations and local races, performed or recorded by Standfuss, he arrives at the following conclusions :—

[1] Compare the facts with regard to *Papilio Sarpedon* given by Jordan, ("On Mechanical Selection," *Nov. Zool.*, 1896, p. 431) in illustration of the position that "abnormal varieties show distinctly the directions in which a species is able to develop". Compare also the result of an experiment cited by Tutt, "Melanism," 1891, p. 15.

[2] Compare the result of an observation by Mr. South, who raised hybrids from the same two forms in 1883 (*Proc. Ent. Soc. Lond.*, 1887, p. xliv.).

1. When the normal form of a species (Grundart) is crossed with a gradually formed local race of the same species, the result is a series of intermediate forms.

2. When the normal form is crossed with a sporadic aberration, the result in many cases is that the issue divides itself sharply between the normal form and the sport, intermediate forms being absent.

Hence, according to Standfuss, the process of species-formation must be gradual; for when two distinct species are crossed, the issue does not split up into the two parental forms as in the case when one parent is a suddenly formed aberration. On the contrary, the behaviour of the issue of two distinct species is very similar in kind to that of a species crossed with a local race or variety which is being gradually established by the accumulation of slight changes. It would seem therefore that although an aberration or sport may be perpetuated by inheritance, it can never acquire distinct specific rank. No doubt however it may, if selected, eventually replace the original form of the species.

To the foregoing considerations it may here be added that these sporadic colour-aberrations seem to have many points of resemblance with the colour-varieties in domestic animals, such as the "lemon-and-white" and "tricolour" of the Basset hounds referred to above (p. 199, note), or the well-known tortoiseshell, tabby and black of cats. The fact that these domestic varieties exist side by side in the same race and even in the same litter, and that true intermediates are rare or absent, seems to suggest that they originally appeared as sports, and that their perpetuation has been ensured or favoured by artificial selection, just as, if Standfuss is right, the dark aberrations of *P. monacha* and *A. betularia* are being perpetuated and multiplied by natural selection. Finally, the well-known case of the "otter sheep" (Darwin, *Variation of Animals and Plants under Domestication*, 1868, vol. i., p. 100), where also intermediates are said to be absent, and other instances on record, show that the phenomenon of sharp division between types in the offspring is not confined to crosses between colour-varieties of the same race, but occurs in other kinds of aberration as well.

F. A. Dixey.

THE NATURAL HISTORY OF IGNEOUS ROCKS: II. THEIR FORMS AND HABITS.

THE diverse forms assumed by bodies of igneous rock are in a general sense familiar to every geologist; but our conceptions of them are to a great extent based upon generalised and conjectural diagrams, and the subject seems to be one worthy of closer inquiry. In the brief review which follows we shall take note more especially of those features which seem to have a bearing on genetic questions, and the various considerations touched on below will thus fall into place in connection with the former article of this series. As we there remarked a close relation between igneous activity and crust-movements, whether of continent-building, plateau-building, or mountain-building type, so we shall have to observe in what follows that the forms and habits of igneous rock-masses are related to the *kind* of crust-movement experienced by the region in which they occur. Other factors are not without important influence on the behaviour of igneous rock-masses, but the connection of the latter with the geological structure of the country is found to be of a very intimate character, extending in many examples to special tectonic details.

While this relation is more evidently verified in the case of intrusive rocks, there are not wanting indications that similar rules govern the phenomena of surface vulcanicity. The association of volcanic foci and of fissure-eruptions with the mountain-type and the plateau-type of structure, respectively, can scarcely be regarded as a fortuitous coincidence. In making a few remarks on volcanic, before passing on to intrusive, rocks we shall, however, draw attention to some other points.

The most obvious factors determining the forms assumed by extruded lavas are the volume of an individual eruption and the degree of fluidity possessed by the material when poured forth; and the latter must depend both on the chemical composition of the lava and on the temperature

at which it is erupted. Experiment shows that viscosity of a molten lava increases with its silica-percentage, especially when the latter exceeds fifty-eight or sixty, and is also increased by an unusual amount of alumina and by even a moderate proportion of potash. Accordingly we find that acid lavas are very decidedly less mobile than basic ones, and so flow less readily and to smaller distances; and further, that certain intermediate lavas, rich in alumina and potash, are remarkably viscous, as is illustrated by the peculiar dome-like forms assumed by some trachytic and phonolitic eruptions. Again, the water which all rock-magmas contain must contribute to reduce their viscosity, though it does not perhaps materially affect the relative viscosity of different types of lavas. In subaërial eruptions much of it doubtless escapes as steam before the lava spreads out as a *coulée*, but in submarine outbursts the necessary retention of the contained water may considerably modify the conditions of flow. It seems probable that, beneath a rapidly consolidated crust, a lava may travel over the sea-floor more readily and to greater distances than it could accomplish on land. In all such questions the extremely low thermal conductivity of rocks, both solid and molten, must be borne in mind.

A temperature considerably above the melting-point is another condition which must promote the fluidity of a lava. In a rock-magma occupying a subterranean reservoir any noteworthy superheating can, as Becker remarks, scarcely be postulated. Unless the magma differs in composition to a remarkable degree from the encasing solid rock, an accession of heat will melt a portion of the latter rather than raise the temperature of the former. This is confirmed by the fact that in almost all lavas crystallisation has begun prior to extrusion. The relief of pressure incident to the extravasation of the magma must theoretically, however, bring about a superheated condition, and direct evidence of this is furnished by the corrosion and resorption of the contained intratelluric crystals. Other things being equal, the degree of superheating will be proportional to the depth through which the molten magma has risen; and

the deeper the source of a lava of given composition, the more fluid will it be when erupted. The same consideration will of course apply to any magma which rises through a considerable thickness of solid rocks, even though it may fail to make its way to the surface.

Information as to the viscosity of modern lavas, derived from observation of their rate of flow, would be of interest. From the record of a basaltic eruption from Kilanea Becker (12) has deduced a viscosity fifty or sixty times as great as that of water. The data available for such calculations seem to be few and wanting in precision, and are confined, of course, to subaërial eruptions. As regards the diminution in viscosity due to relief of pressure, the experiments of Barus go to minimise its importance. He finds that the diminution of pressure must be at least 200 atmospheres (or say 2400 feet of rock) to reduce viscosity as much as one degree rise of temperature would do.

A few words should be said here of the roughly cylindrical mases, sometimes of solid igneous rock, sometimes of fragmental accumulations of material, either volcanic or non-volcanic, which mark the actual vents through which volcanic discharges have reached the surface.[1] Of these so-called *necks* many examples are preserved in the districts of former volcanic activity in this country, and illustrations of numerous cases are given in the two handsome volumes recently brought out by Sir A. Geikie (13). The supposition that the conduits or pipes represented by these bodies have been formed by violent explosive agency is one obviously prompted by the observed facts, but it is of interest to find confirmation of it from a new line of research, as developed in certain memoirs by Daubrée (14). The typical instance considered by him is that of the well-known necks in the diamond-fields of South Africa. Seventeen of these are known situated on a straight line some 125 miles long. Each of these has a generally

[1] In a recent paper Captain A. H. McMahon notes on the northern border of Baluchistan a "huge natural pillar" of trachytic ash having a diameter of 100 yards at the base and rising over 800 feet (*Quart. Journ. Geol. Soc.*, vol. liii., p. 293, 1897).

cylindrical form, the width usually diminishing downward. The bounding walls exhibit a remarkable vertical striation. The strata traversed have experienced no alteration at the contact, except that they are upturned. The contents of the pipe are fragmental materials, mainly an ultrabasic breccia, which is the matrix of the diamonds. The phenomena here summarised have been imitated experimentally by Daubrée by the use of high explosives, and his results are, as he shows, of great geological importance. Their application is not only to the drilling of cylindrical apertures, but also to the comminution of rocks to produce volcanic dust, the apparent plasticity of rock-masses under mechanical forces, the upthrust of plugs of solid rock through vertical perforations, and other geological phenomena. The author named believes that some trachytic domes must have been erupted in a nearly solid—not even pasty—condition. In any case the connection between "necks" of breccia or agglomerate and those consisting of plugs of solid rock is a close one, the latter being doubtless due in many instances to a neck formed of fragmental materials having subsequently been invaded by an uprush of molten magma. The essential feature of a true neck, as contrasted with the plug-like intrusions to be mentioned below, is that it represents what has been an actual channel of communication between the interior and the exterior of the earth's crust.

Proceeding then to the forms of intrusive rocks, we consider first, and more particularly, those characteristic of regions where the disturbances of the earth's crust have not been of a kind involving lateral thrust. The intruded sheets, conveniently designated *sills*, are not confined to areas exhibiting this type of structure, but they certainly attain their highest development and show their greatest regularity among flat strata, or more accurately, among strata which have not experienced any marked folding referable to the period of the intrusions or earlier. One obvious reason for this is that folding would tend to seal the original partings of the strata in the limbs of the folds, while concurrent fracture would open an easier channel for the molten magma. The absence or rarity of dykes in many

districts of regular sill-formed intrusions is in accord with this. The sills themselves have presumably been fed by dykes, but these are in the nature of the case rarely exhibited. Again, the sill form seems to be related also to the composition of the magma so injected. It has been remarked by more than one writer that typical sills are usually of basic rocks, and this observation seems to connect itself with the greater fluidity of basic as compared with acid magmas. The thickness of the "cover" may also perhaps influence the form assumed by an igneous magma injected among stratified rocks. Russell (15) gives reasons for believing that sills have in general been formed at no great depth below the surface at the epoch of the intrusion; and this he explains by the consideration that, the less the energy expended in lifting the overlying rocks, the more will be available for effecting lateral expansion. There must of course be a limit to the operation of this rule, since if the pressure above be insufficient to restrain the fluid magma, the latter will break out. What this limit may be, we cannot at present state; but certain observations, to which reference has been made in a former number of this journal,[1] indicate that under submarine conditions it may be very small. Certain basic sills in Cornwall and in California, showing curious curvilinear divisions, have been supposed to represent injections effected among deep-sea sediments very near to the ocean-floor. Geikie (13) has recently pointed out the wide distribution of this "pillow-structure" among certain basaltic and andesitic rocks in Britain, especially in the Ordovician of the Ballantrae district and Forfarshire, and of Tyrone, Lough Mask, and other parts of Ireland. He ascribed it to the lavas having flowed into water, but in some instances (*e.g.*, at Cader Idris) the hypothesis of intrusion seems also to be a tenable one. In most cases associated radiolarian cherts give evidence of thoroughly deep-sea conditions.

These various points come out more clearly when the characteristics of sills are compared with those of laccolites.

[1] SCIENCE PROGRESS, vol. v., p. 480.

The typical *laccolite*, using the term in its original and strict sense, is associated with the plateau, not the mountain, kind of structure, and the most perfect examples of it have been described from the great plateau region to the west of the Rocky Mountains. Its special feature, as contrasted with the other types of intrusion to which the name laccolite has been extended, is that the strata beneath are undisturbed, while those above are elevated into a dome, this elevation being due directly to the intrusion itself. The simple laccolite thus differs from the uniform sill in its more restricted area and greater relative thickness at its centre, the lenticular mass thinning out rapidly in every direction. In other words, the elevation of the overlying strata, instead of being extended and of small vertical amount, is localised and correspondingly accentuated. The facts elicited above lead us to inquire whether this difference is connected with a higher degree of viscosity in the magma and a greater thickness of superincumbent strata. As regards the former condition at least the answer seems to be in the affirmative, the typical laccolites being of acid and intermediate rocks. Those of Colorada, Utah and Arizona, as described (6) by Whitman Cross, have silica-percentages ranging from 56·62 to 73·50, with an average of 63·84 : the percentage of potash varies between 1·97 and 3·95, the average being 3·02. Rock-magmas of such composition would be much more viscous than the diabases and basalts which are so familiar in the form of sills. It is true that Gilbert (16) has recorded laccolites at Twin Butte, in the south-eastern part of Colorado, composed of what he characterises as a basic syenite-porphyry, but no account of this rock has yet been published,[1] and the limited exposures make the form of the intrusions a matter of inference rather than demonstration. The author named rejects any generalisation as to the connection of the laccolitic form with the composition of

[1] If this is the rock from "Two Buttes," of which an analysis has since been given (*Bull. No.* 148, *U.S. Geol. Sur.*, p. 182, 1897), it seems to be allied to tinguaite. Though poor in silica, it contains 4·08 per cent. of potash and 6·70 of soda, and its magma would doubtless possess a high degree of viscosity.

the rocks, and suggests that basis laccolites may have been overlooked owing to their not giving rise to salient topographical features; but the facts, so far as they are known, seem to point to a more positive conclusion. Indeed Gilbert in his original memoir on the Henry Mountains laid stress on "the coincidence of the laccolitic structure with a certain type of igneous rock," and based on this his theory, which can scarcely be maintained at the present day, that the behaviour of the intrusions depended upon "the relative densities of the intruding lavas and of the invaded strata". As regards the question of load, it seems to be generally agreed that typical laccolites were in all cases formed under a very heavy cover of overlying strata. The stratigraphical evidence pointing to this conclusion is reinforced by the results of Cross's petrographical study of a large suite of specimens. Another point noticed by Gilbert in the Henry Mountains is an apparent relation between laccolites and the nature of the encasing strata: they are always enclosed by soft beds. This is, however, to be considered not so much a condition of the formation of laccolites as a factor in determining their selection of a horizon. It does not apply to the associated sheets and dykes.

Excluding the modifications observable in laccolitic intrusions among strata either folded or in process of folding, there are still occasional departures from the simple form of the regular dome with flat base. One such special development is seen when the intrusive mass has spread, not along one, but along many bedding-planes, the central mass passing laterally into a number of rapidly thinning sheets. Instead of the ideal mushroom form, there results one which is comparable rather with a cedar-tree. This, according to Holmes, is exemplified in the La Plata Mountains of Colorado, and Geikie has drawn a similar section to represent the relations of gabbro masses injected among the bedded basalts of Skye. In the latter case a connection seems to be suggested between the partial reversion to the sill form and the basic nature of the intruded magma, but the relatively acid composition of the La Plata rocks forbids our laying much stress on this point.

To the sill and the laccolite Russell (15) has added another type of intrusion, to which he gives the name *plutonic plug*, constituting the third term of a genetically related series. As the laccolite differs from the sill in the narrower localisation of the intrusion and of the resulting uplift of the overlying strata, so in the plutonic plug this localisation is carried to an extreme degree, and the form of the igneous rock-body becomes cylindrical rather than dome-shaped. The intrusions taken as typical examples occur in Wyoming and South Dakota, where they have given rise to a number of hills, some having very striking outlines. The hill named Mato Tepee is of columnar shape, rising almost vertically to a height of more than 600 feet above the platform at its base, and composed of igneous rock thrust like a plug through the strata of the district. The latter are horizontal and undisturbed, even up to the very base of the tower. Other examples are more or less completely exposed, and in some localities, as at Little Sun Dance Hill, there is only a dome-like elevation of the strata to indicate a plug entirely concealed. Comparison of the different cases makes it clear that the igneous magma never reached the surface, and in other respects the intrusions differ entirely from volcanic "necks". The texture of the igneous rocks gives evidence of consolidation at a considerable depth, and the geological relations of the district warrant the same conclusion. The arching of the strata over the concealed plug of Little Sun Dance has been effected without fracture, which argues a very great superincumbent load, and the absence of dykes throughout the region is significant in the same sense. As regards the petrographical character of the intrusions, there is some obscurity in Russell's account, but the rock of Mato Tepee has recently been examined by Pirsson. It is of phonolitic composition, with 61·08 per cent. of silica and 18·71 of alumina. We may safely infer that the magma was of a highly viscous nature, probably comparable with those which in volcanic outpourings formed the trachytic lava domes of Auvergne and the phonolitic protrusions of that and other districts, to which may be added the curious puy-

like domes rising through and above level flows of leucitophyre and leucitite in the Leucite Hills of Wyoming, recently described by Kemp (17).

Returning to the laccolite, it may be observed that even in the High Plateaux of the West, as the Rocky Mountains are approached, the regular lenticular form of intrusion gives place to what has been termed an *asymmetric laccolite.* This is seen in Mount Marcellina and other examples in the West Elk Mountains of Colorado. The intrusion of Mount Axtell is described as "a sheet greatly thickened at one extremity". In some cases such a form is seen to stand in direct relation with a sharp monoclinal fold, which may pass into a fault in an upward direction. Here it is no longer possible to regard the disturbance of the strata as the direct result of the igneous intrusion, but we may perhaps consider the folding and the intrusion as closely connected results of the same set of forces in the earth's crust. In the Rocky Mountain belt itself, for instance in the Leadville district of Colorado, as described by Bulkley (18), we go a step farther. The Palæozoic strata are here thrown into large folds, in which occur intrusive bodies of rock comparably petrographically with the typical laccolites of the High Plateaux to the west. To the lateral thrust which has affected the strata must be ascribed not only the folding, but also the formation locally of gaping cavities along the bedding-planes, into which the igneous magma has been forced. It is not probable that actual vacant spaces were ever formed, the inflow of the magma being presumably concurrent with the process of folding. If, however, we are to separate these two results of a common cause, we must regard the disturbance of the strata as the occasion of the intrusion, not as its consequence.

From the point of view just indicated we perceive an essential distinction between the original laccolites of Gilbert and the lenticular intrusive bodies in *folded regions* to which the title has been extended by some writers. With reference to the disturbance of the associated strata, the one type is an antecedent, the other a consequent phenomenon. A distinctive name for the latter type is still a desideratum.

The nature of the intrusion itself and its relation to folding were made clear by Lossen (19) in his researches in the Harz district, and have been generally recognised. Examples of lenticular intrusive bodies of diabase following the bedding of the strata and clearly determined by folds have been pointed out by Watts at Corndon and other places in the Shropshire district. In some parts of North Wales the maps of the Geological Survey show very evidently how the diabases have been injected mainly along the crests and troughs of the folds, that is, in the places where the strata would tend to part in consequence of the lateral thrust. Here the individual intrusions attain no great thickness, being rather of the nature of inconstant sheets. Regarding the more massive lenticles as the analogue in a mountain-region of the typical laccolite, these more attenuated forms correspond with those which in a less disturbed region appear as regular sills. Among folded strata, however, the relative thickness and extent of an intruded mass cannot have the significance which has been ascribed to them in a plateau region, being determined by externally impressed forces rather than by the fluidity of the magma or the depth of cover. It should be observed too that the surfaces of weakness in the strata which guide the injected magma are not always those of bedding, but may be determined by faults, overthrust or underthrust faults, or other structural accidents.

The varied bodies of intrusive igneous rock hitherto referred to are of well defined and clearly ascertainable forms, but the same cannot be said of the masses, often of considerable size, known as *bosses* or *stocks* and consisting generally of rocks of plutonic characters. These large intrusive masses are, as a rule, clearly transgressive as regards their exposed upper surfaces, but the form and relations of their deeper parts are necessarily matters beyond direct observation, and very divergent opinions have been held concerning them. Some geologists have considered a boss, say of granite, as exposed at the surface, to be merely the summit of a great mass, pictured as of roughly pyramidal shape, extending downward with increasing horizontal di-

mensions to an indefinite depth. Others regard an ordinary granitic intrusion as differing only in its greater size and less regularity from other intrusive bodies which can be more completely examined, and comparable in its general shape and relations with a sill or laccolite, or in some cases perhaps with a "plug". The question is closely connected with another one, *viz.*, to what extent a rock-magma under plutonic conditions "assimilates" or incorporates by fusion portions of the neighbouring solid rocks. One extreme view is that intrusive rocks in general have replaced rather than displaced the rock-masses which they have invaded. Pushed to its limit, this hypothesis leaves little or no room for any invading magma at all, and therefore implies that the igneous rock has been derived almost wholly from the fusion *in situ* of pre-existing solid rocks. Such a supposition seems to be scarcely tenable in the case of an igneous rock-mass occupying a definite space among surrounding rocks and encircled at most by a limited aureole of metamorphism. It is difficult to believe that a relatively small amount of intruded magma could carry sufficient heat to melt a large bulk of rocks, and equally difficult to account for the narrow localisation of heat if supposed due to other causes. That a molten rock-magma may to some extent and under suitable conditions melt or dissolve its encasing walls and fragments detached from them is well known, and observations which may be referred to on another occasion seem to indicate that in certain cases this action is of considerable importance, but its application is probably very limited. There is no doubt that a channel once formed for an igneous intrusion may be enlarged in this and other ways, the magma penetrating fissures and detaching fragments, which are partly dissolved, partly carried forward or upward. Perhaps something of this kind has occurred, for example, in the Ross of Mull granite as described by Goodchild (20), but such an action cannot be described as an assimilation of the surrounding rocks by the granite magma.

The question may obviously be approached from the chemical side, and here the evidence is decidedly against

"assimilation" as a factor of importance in the making of plutonic or other igneous rocks in general. Any considerable incorporation of quartzites or grits should give rise to an igneous rock of much more acid composition than any with which we are acquainted, and similarly the taking up of calcareous rocks should show itself in an abnormal content of lime. As regards the latter, Brögger (11) takes the instance of a large granite intrusion among the Lower Palæozoic formations of Southern Norway. The strata, which abut on the granite, and are truncated by it, are accurately known; most of them are calcareous, and it is calculated that the mixture obtained by melting them together in the proper proportions would have $24\frac{1}{2}$ per cent. of lime. The granite, however, contains only $\frac{1}{2}$ per cent. This is not too large an amount for an ordinary granite; but even assuming the original magma to have been free from lime, it is seen that it cannot have taken up more than one forty-eighth part of its own mass from rocks like those seen in contact with it. Brögger asks, what then has become of the prolongation of the strata cut off by the granite? Since the portions cut off have not been incorporated in the magma, they must exist beneath or beyond the granite. In other words the, granitic body must have a concealed under surface, and be an irregular sheet or lens. The argument is evidently one not restricted to the particular case discussed.

We shall conclude these notes on the morphological characters of igneous rock-bodies by drawing attention to certain peculiarities well exhibited by the Tertiary dykes and sills in the Western Islands of Scotland (13, 21).

One noticeable feature is the tendency of these igneous intrusions to follow lines already marked out by earlier intrusions of like material, and probably from the same source. In this way arise *multiple* dykes and sills. Many basalt dykes in the volcanic area of Skye, for instance, are not simple bodies corresponding in each case with a single injection of the basic magma, but are double, triple or perhaps tenfold, consisting of several distinct dykes in juxtaposition and representing as many successive injections. Not in-

frequently this can be verified, when the edges of the component dykes are indicated by compact selvages, bands of amygdules, etc.; and we may suppose that other cases are overlooked in the absence of such decisive evidence. Apparent bifurcation is often due to the separation of two dykes or sills which for some distance have run in contact with one another.

We have assumed the several injections which go to build up a multiple intrusive body to belong to a single rock-type, but mention must also be made of the modifications in form and habit produced by the association of different rock-types in *composite* multiple dykes and sills. Good illustrations are again afforded by the Tertiary rocks of the British province, which include both acid and basic types. In the larger intrusive bodies these are represented by granite, often with a micrographic tendency, and gabbro, graduating into ophitic diabase, etc. It is well known that the two rocks occur together in the Carlingford district, in Arran, Mull and Rum, in the central part of Skye, and as far away as St. Kilda, and always in close association. In the dykes and sills, however, the relations of the two corresponding rocks—granophyre and basalt—are sometimes of a more intimate nature. In particular, Sir A. Geikie has drawn attention to a remarkable group of composite sills intruded at various horizons in the Lias of Skye, in which the acid and basic rocks exhibit a curiously symmetrical arrangement. Usually there is a central and thicker sheet of granophyre, having in contact with it above and below two thinner sheets of basalt. These composite triple sills are not due to "differentiation in place," but the acid magma has found its way between two basalt sheets which previously constituted a "double sill". More complex cases are also met with.

Triple dykes showing a similar association are not infrequent in Skye, and Geikie noted one consisting of a central dyke of spherulitic granophyre, eight or ten feet wide, flanked by two narrower dykes of basalt. Judd had already remarked similar cases in Arran. One at Cir Mhor, which was specially studied, has a central part of

pitchstone-porphyry and quartz-felsite between two dykes of augite-andesite. In an earlier paper by Judd (1874) we find a note recording a section at Mingary Castle, Ardnamurchan, where "a mass of quartziferous porphyry has been forced between beds of Lower Lias shale, while a later-formed sheet of basalt has evidently taken advantage of the plane of weakness, constituted by their junction, to force itself between them". The author compares this with the case of Puy Chopine in Auvergne, described by Scrope and others.

At certain localities in Skye composite sills of the sandwich-like type referred to seem to be in connection with composite dykes, which have probably been their feeders. It appears thus that the successive basic and acid intrusions have not only spread along the same bedding-plane, but reached that position by the same channel; while facts might be cited pointing to an interval of no great duration between the two injections. Such considerations lend some support to the idea that the two magmas, prior to intrusion, existed together in a common subterranean reservoir. If this idea be granted, there is only a step from successive intrusions of different magmas to a single intrusion of a heterogeneous magma. We cannot premise with any certainty the behaviour of an intruded magma drawn from different portions of a reservoir already differentiated; but phenomena familiar to all geologists clearly point to the existence of what may be termed *heterogeneous* intrusions. An example involving, not acid and basic rocks, but more or less basic varieties of gabbro, has been studied by Geikie and Teall in the Isle of Skye.

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ALFRED HARKER.

GERMINATION OF SEEDS: II. THE HYDROLYSIS AND REGENERATION OF PROTEINS.

THE chemical processes that obtain during germination have been submitted to extensive examination; this application is certainly well deserved, for there is no physiological study that is more likely to elucidate the immensely complex changes that are inseparably connected with phenomena of vegetable life. In the seed is a large store of plastic material which is entirely consumed during development of embryo into seedling, and seedling into plant, so that exceptional facility is granted to all that would ascertain, the manner in which the plant renders this material available for the synthetic transformations to which it is afterwards subjected, as well as the nature of these changes themselves. Since there is no reason for doubting, that the metabolism of germination is, in the main, chemically similar to that metabolism which occurs in mature plants throughout the whole vegetable kingdom, it is almost unnecessary to indicate the superlative importance that is attached to investigations bearing on this question. If the present knowledge of this subject is still deficient in many points, this is rather due to the very considerable difficulties with which the question is beset, than to poverty of research. That which is known of these processes, is very largely due to the most excellent work that has been produced by E. Schulze, and the school of investigators that has emerged from his laboratory at Zürich.

Before the question of proteïnic transformations is entered upon, it is advisable to discuss the system of proteïnic nomenclature, that seems to be now prevalent in this country. This is both deficient and redundant; for, although the term "proteïn" is used to connote the whole class of compounds more or less resembling the albumins, it is commonly replaced by the word "proteïd," which serves also to designate the group of still more complex

substances, such as hæmoglobin, nucleïn, etc. Futhermore, the term "albuminoid" is indifferently utilised to connote both that group of compounds centred round the albumins, and also that associated with gelatin or keratin. The incongruity of this system is obvious, and any attempt to replace it with one more compatible with modern requirements can only be desirable.

A. Wròblewski[1] has recently published a modification of Drechsel's classification, which is adopted in the following pages. The term proteïn is employed by him in widest connotation; he divides proteïns into three classes—(i.) "Eiweissstoffe," (ii.) "Zusammengesetzte Eiweissstoffe," and (iii.) "Eiweissähnliche Substanzen". Although these divisional names may suffice in Germany, it is impossible to effect a concise translation that is satisfactory. It is hence suggested that "Eiweissstoffe" be rendered as "albuminoids," which plan has at least the virtue of rational application, and "Zusammengesetzte Eiweissstoffe" as "proteïds," in which sense the word was employed by its inventor, F. Hoppe-Seyler. But no expression is then left for "Eiweissähnliche Substanzen"; this class is only a refuse-heap into which compounds are swept that will not fit into the other two, and to find a suitable expression is difficult: it is indeed impossible to find any to which many objections could not be made. But since for practical purposes some collective term is necessary, it is suggested that, since gelatin is a prominent member, the word "gelatoids" be provisionally used; there is at least a precedent in the designation "alkaloid".

Wroblewski excludes protamines from proteïns, whereas A. Kossel includes them, and inserts proteoses and peptones, which Neumeister has eliminated.

The reserve-proteïns of hypnotic[2] seeds are as a rule chiefly in the form of aleurone-grains. This term is used very widely to signify all proteïnic granules that are neither living nor hypnotic. The grains are either more or less

[1] *Ber. d. deut. ch. Ges.*, xxx. (1897-8), p. 3045.

[2] For the application of this term, see SCIENCE PROGRESS, N.S., vol. i., No. 5, p. 605.

homogeneous optically, or contain inclusions. The latter are principally proteïnic crystals, the crystalloids, or such as are usually spheroidal, the globoids; crystals of calcium oxalate also occur occasionally. The globoids may possibly consist of the compound or mixture described by Winterstein,[1] which is the calcium-magnesium salt of an acid phosphoric ester, that yields inosite on decomposition. The aleurone-grains are embedded in a proteïnic matrix; both may suffer dissolution during germination, in which case the matrix is composed of necroplasm,[2] this being the case in certain endosperms; or only the grains may be thus affected, when the matrix consists of hypnoplasm,[3] as is the case in the cotyledons of seeds. Previously to dissolution the substance becomes swollen and hyaline.

Inasmuch as appreciation of the various theories, founded on the occurrence of certain nitrogenous compounds during germination, requires knowledge of the products of artificial proteohydrolysis, it is perhaps advisable to devote a few words to the subject.

The question of artificial proteohydrolysis has been attacked by several observers.[4] As a result of their investigations, it is known that the products fall principally into three groups—(1) nitrogenous bases, (2) aliphatic amido-acids, and (3) aromatic amido-acids. Ammonia is also formed. Examples of the first group are—lysin (diamido-caproic acid), lysatinin (a compound creatin), arginin (guanido-*a*-amido-valerianic acid), histidin. Examples of the second group are—aspartic acid (amido-succinic acid), glutaminic acid (*a*-amido-glutaric acid), *a*-amido-valerianic acid, and leucine (*a*-amido-caproic acid). The third group is exemplified by—phenyl-*a*-amido-propionic acid and tyrosine (para-hydroxyphenyl-*a*-amido-propionic acid). Several other products have been obtained, but they have at present no demonstrated connection with the subject under dis-

[1] *Ber. d. deut. ch. Ges.*, xxx. (1897), p. 2299.

[2] See SCIENCE PROGRESS, *loc. cit.* [3] *Ibid.*

[4] Notably by Hlasiwetz and Habermann, Horbaczewski, Schützenberger, E. Baumann, E. Salkowski, Nencki, E. Drechsel, A. Kossel, E. Schulze, S. G. Hedin, and others.

cussion. Different groups of albuminoids and gelatoids yield different products; thus, gelatin, etc., bases and aliphatic amido-acids; peptones, etc., bases, aliphatic and aromatic amido-acids; and proteïns containing sulphur give rise to the same substances as peptones, but also to sulphur-compounds. The protamines are distinguished through yielding only bases.

Proteïds yield albuminoids and various other substances on hydrolysis; of these, nucleïns yield the purine-bases (hypoxanthin, etc.) and nucleïnic and thyminic acids.

The nucleïns and nucleoproteïds are characteristic constituents of nucleoplasm generally, and protamines of that of spermatozoa, whereas in plants the albuminoids and certain gelatoids are chiefly found in the cytoplasm.

The occurrence of asparagin in germinating seeds and seedlings has been long known. It was first discovered by Vauquelin and Robiquet (1805) in the shoots of *Asparagus officinalis;* in 1858 Th. Hartig ascribed to it a general distribution in plants, as did also Boussingault, which statement has been since amply confirmed.[1] The former also regarded it as the form in which the metastasis of proteïns is effected. In 1872 Pfeffer opposed the view of its general distribution, affirming that it was only produced during germination; he too supposed it to be the metastatic form of reserve-proteïns. This view would seem to obtain general acceptance among phytologists of to-day, although it has long been refuted, through the works of E. Schulze and his collaborators, in the form in which it was then promulgated by Pfeffer.[2]

Von Gorup-Besanez was the first to show that amido-compounds other than asparagin occur in etiolated seedlings of *Vicia sativa*, and his results have been confirmed and extended, a whole series of nitrogenous substances having been discovered that can be regarded as products of proteohydrolysis during germination. The observations have been chiefly made on etiolated plants, because more

[1] For literature on asparagin previous to 1882, see *Die Pflanzenstoffe*, Husemann & Hilger, Berlin, 1882, p. 264.

[2] Pfeffer does not now retain his old opinion.

favourable material is thus obtained for quantitative determinations, inasmuch as darkness has a considerable retarding influence on the metabolism of nitrogenous compounds. Since a description of the methods of analysis adopted in these investigations would be out of place here, the publications concerned with this question are included in the bibliography and will receive no further notice.

In the subjoined table is a list of some of the plants that have been more systematically examined, with the nitrogenous compounds resulting from proteohydrolysis during germination that have been isolated and identified, as well as with a notice of the etiolated or chlorophyllous condition of some of the seedlings. It has been found that the effect of insolation is to materially alter the quantity in which these bodies are found in etiolated seedlings; this alteration is in cases so extreme as to effect a qualitative difference :—

Plant.	Products of Proteohydrolysis.	
	Etiolated.	Chlorophyllous.
Picea excelsa.	Arginin, Asparagin, Glutamin.	Arginin, Glutamin.
Pinus sylvestris.	Ditto.	
Abies pectinata.	Arginin.	
Vicia sativa.	Asparagin, Glutamin, Phenylalanin, Butalanin, Leucine.	Asparagin, Leucine.
Lupinus luteus.	Asparagin, Phenylalanin, Butalanin, Arginin, Leucine.	Leucine, Arginin.
L. alba.	Asparagin, Phenylalanin.	Leucine.
L. angustifolius.	Leucine, Butalanin.	
Soja hispida.	Asparagin.	
Cucurbita Pepo.	Glutamin, Asparagin, Tyrosine, Leucine, Arginin.	
Ricinus communis.	Glutamin, Arginin.	

For the sake of convenience, amido-valerianic acid and phenyl-amido-propionic acid are designated by their shorter names, butalanin and phenylalanin, in this table.

The occurrence of these compounds is certain, all having been isolated and identified, both qualitatively and quan-

titatively, according to strictest chemical canons. But in addition to these there are others, the occurrence of which is very probable, but which are not found in sufficient amount to enable their identity to be irrefragably established. Thus, tyrosine is most probably present in *Vicia sativa* and *Lupinus luteus* (etiolated); amido-valerianic acid in *Lupinus alba* (etiolated and chlorophyllous) and *Soja hispida* (etiolated); phenyl-amido-propionic acid in *Lupinus alba* (chlorophyllous), *Soja hispida* and *Lupinus angustifolius* (etiolated); also leucine and arginin (or similar base) in etiolated *Soja hispida.*

The quantitative relations existing between these compounds will be discussed below, but it may be advisable to mention that glutamin replaces asparagin in certain plants,[1] whereas asparagin replaces glutamin in others,[2] also that in three of these glutamin and asparagin occur together.[3] These plants contain also in small amount some of the amido-acids, tyrosine, phenyl-amido-propionic acid, amido-valerianic acid and leucine.

That these compounds result from chemolysis of the reserve-proteïns is certain. For, it has been quantitatively established that their formation is correlated with loss of proteins, and that the N-content of amido-compounds and proteins are at any given period complementary. Further, it has been satisfactorily shown that, although in some cases very small amounts of amido-compounds, nitrogenous alkaloids, and other nitrogenous bodies of unknown nature, occur in those seeds from which seedlings have been reared, the amount of these is wholly insufficient to yield even a small fraction of the substances in question. Again, the preparation of extracts and isolation of the compounds has been so undertaken, that their origin could not be accounted

[1] *Ricinus communis. Sinapis alba. Brassica Napus*, var. *annua. Lepidium sativum. Raphanus sativa*, var. *radicola. Camelina sativa. Spergula arvensis. Spinacia glabra.*

[2] *Triticum vulgare. Zea Mays. Lolium pratense. Arrhenaterum elatius. Phleum pratense. Papaver somniferum. Tropaeolum majus. Pinus sylvestris. Picea excelsa. Helianthus annuus. Cucurbita Pepo.*

[3] *Helianthus annuus. Cucurbita Pepo. Picea excelsa.*

for, either through reactions connected with the process, or through putrefaction. Moreover, since these bodies have been obtained without exception from artificial proteohydrolysis, it is inconceivable that they can have arisen in any other manner than by transformation of reserve-proteïns, especially since the seedlings were allowed to derive their nutriment solely from these.

There is one point of difference between the products of proteïnic decomposition during germination and that in vitro, namely, that, whereas aspartic and glutaminic acids are produced in the latter, their amides occur in the former, the acids having been never observed in plants. But the difference is a necessary consequence of the dissimilar conditions and only apparent, for in artificial hydrolysis ammonia is formed, this having arisen from the amides which could not exist in the conditions.

Besides these products two other nitrogenous substances have been found in certain seedlings, namely allantoïn (glyoxalyl diureïde) and guanidin (imido-urea), that result probably from proteïnic decomposition. Allantoïn was found by C. Richardson and C. A. Crampton in germinated seeds of *Triticum*, and by E. Schulze and some of his collaborators in germinated buds. Guanidin occurs in etiolated seedlings of *Vicia sativa* and possibly in chlorophyllous ones. Inasmuch as arginin is guanido-α-amido-valerianic acid, it is possible that the guanidin found in *Vicia sativa* may arise from that part of the proteïnic molecule, that would yield arginin in other circumstances.

The series of nitrogenous compounds just described account more or less for the carbon, hydrogen, nitrogen and oxygen of the proteïnic molecule ; it is true that there almost certainly remain some substances composed of these elements not yet separated, but if these are not known in an isolated condition, the class to which they belong is known. But the sulphur still remains to be accounted for. There can be no doubt but that this is finally found in sulphates, for E. Schulze and his collaborators have shown that sulphates are formed during germination of *Vicia sativa*, *Lupinus luteus*, and *Cucurbita Pepo*, and that there is an increase

on the content of sulphates of the hypnotic seeds. The increase is greater in *Lupinus*, as proteïnic chemolysis is more energetic here, and the reserve-proteïns are richer in sulphur. Since the amount of sulphates formed in *Lupinus* approximated finally to that theoretically obtainable from the proteïns, it was concluded that the latter formed the source; but inasmuch as other organic S-compounds were present in the hypnotic seed, capable of yielding the sulphate formed, *Vicia* and *Cucurbita* were examined, and an increase correlative with proteïnic decomposition established. It was noticeable that in *Lupinus* the increase of sulphates during earlier stages of growth did not correspond with that theoretically possible, approximation occurring later; this indicates formation of antecedent thio-compounds. Tammann has found traces of acid sulphuric esters in seedlings, but not more than in seeds; it would be a matter of very considerable interest to ascertain whether these are formed, or perhaps thio-oxyacids.

Nucleïns and nucleoproteïds have been shown by A. Kossel and others to yield purine-bases on hydrolysis, and they have found that these bases have a wide distribution among animals and plants. The production of these substances from plants was first noticed by Schützenberger, who found them in yeast subjected to putrefaction. Their presence has also been demonstrated in seedlings, but the chemical identity of the separate bases occurring has not been satisfactorily determined in all cases, mention being usually made of purine-bases, either because establishment of their nearer nature has not been attempted, or owing to difficulties connected with this. They have been found in etiolated seedlings of *Lupinus luteus* and *Cucurbita Pepo*, young green plants of grass, of *Trifolium pratense*, of *Avena* and *Vicia sativa*, of etiolated *Soja hispida* and malt. Guanin occurs in *Vicia sativa* and *Cucurbita Pepo*, hypoxanthin in *Lupinus* and probably in *Cucurbita*, xanthin probably in *Lupinus*.

The proof that the purine-bases are in these cases existent as such in the material, having been derived from the proteïds contained in the reserve-organs, and not from

those in the growing plant, is not quite satisfactory. For Kossel has shown that boiling water decomposes nucleïns gradually, so that the bases found may have been formed in the preparation of the extracts. But it has been ascertained that the lecithins of many seeds diminish during germination, whereas cholin and phosphates increase, indicating decomposition of the former. It may be that the phosphorus of the lecithins is derived from nucleïns, and since nucleïns are not rapidly decomposed through boiling water, there is a probability that the proteïds of the reserve-organs are utilised during germination.

Salts of ammonium are only formed in small amount and do not appear to play any part in the hydrolysis and metastasis of proteïns. The same is true of nitrates, which are not normal constituents of germinating seeds.

Although the nitrogenous compounds are subjected to such considerable alteration during germination, it has been proved[1] that the N-content remains constant, so that there can be no loss of nitrogen through excretion or formation of volatile compounds.

It has been assumed once or twice thus far, that the proteïnic chemolysis of germination is hydrolysis. The assumption was, however, but an anticipation, for there can be no doubt that the reserve-proteïns are dissolved through hydrolysis and not oxidation, or any other conceivable process. The proof of this is deducible from the following considerations: The artificial chemolysis of proteïns that gives rise to the various compounds mentioned above is hydrolysis, the reaction being effected either through heating the proteïns with strong hydrochloric acid in the presence of stannous chloride, or through the action of barium-hydroxide at high temperatures in a sealed tube. But the products of the proteïnic chemolysis of germination are without exception also found among those resulting from artificial proteohydrolysis. Although many compounds that are produced in the artificial treatment have not yet been demonstrated in seedlings, this does not prove that

[1] By Boussingault, Sachsse, Leclerc, Behrend and Wilsing, Schulze and others.

they do not occur, for they may be present in such small quantities as to defy isolation, or the plant may consume them as soon as they are formed. Also, some of the artificial compounds may not be primary products, but due to secondary reactions. Moreover, the products that result from the oxidation of proteïns *in vitro* are not those found in seedlings, although closely connected in some cases, as might be expected. If, too, the process were one of oxidation, it is difficult to see why the increase of sulphates in the early periods of germination of *Lupinus luteus* should not correspond with the amount theoretically obtainable from the decomposed proteïns, this approximation being only acquired in the later stages. These facts are quite explicable, if the process is one of hydrolysis. Further, Pfeffer, has stated, that if the reserve-proteïns are completely converted into asparagin, a residue of carbon and hydrogen must remain, since asparagin has a percentage-composition that is richer in nitrogen and poorer in carbon and hydrogen than proteïns. Now Palladin and Löw regard the matter in this light, and assume that these residues are oxidised to form carbohydrates, which Palladin regards generally as products of proteïnic oxidation. But Prianischnikow, relying on the results obtained by Schulze and himself with *Vicia sativa*, shows that, through subtracting from Lieberkühn's formula for proteïn, of which Palladin makes use, an approximate computation of the total amount of substance formed from reserve-proteïns in this case, there is left a small residue of carbon, that need be only hydrolysed to form carbohydrates. Thus the objection of Löw and of Palladin falls to the ground.

But the view of hydrolysis is still further strengthened through observation of the occurrence of proteäses in germinating seeds, which exercise *in vitro* a hydrolytic action qualitatively similar to that of pepsin.

When these considerations are justly weighed, but one conclusion can be made, namely, that of Schulze and Prianischnikow, that the process through which reserve-proteïns are converted into amido-compounds is hydrolysis, not oxidation.

The position has now been reached in which it is desirable to discuss the agency through which the proteo-hydrolysis is effected. In 1874 von Gorup-Besanez stated that he had extracted proteäse from several seeds and kiln-dried malt, capable of converting fibrin into peptone. Shortly after Krauch criticised the methods of von Gorup-Besanez, and instituted experiments which in every case gave negative results. About the same time Schulze claimed to have found peptones in the seedlings of *Cucurbita Pepo* and *Lupinus luteus*, the quantity being very small. The knowledge was, however, at that time insufficient to enable discrimination between proteoses and peptones to be effected; the seedlings may have hence contained either or both. More recently Reynolds Green has attacked the subject, and stated that a proteäse exists in seedlings of *Lupinus hirsutus* and *Ricinus communis* resembling trypsin in its hydrolytic intensity. The proof adduced in support of this is not, however, quite convincing. The most truly satisfactory work on this subject is that of Neumeister, who has lately criticised his predecessors, and examined a large number of seeds and seedlings. He was unable to discover a proteäse in any hypnotic seed, but found one in seedlings, although not in all. Wherever proteäse was found peptones were also demonstrated, but these occurred also in seedlings in which no proteäse could be detected. This was found to be due to pre-existence of peptone in the seeds of the latter. The amount of proteäse and peptones demonstrable varied in different species. The proteäse proved to resemble pepsin in so far as the hydrolysis ceased after peptonisation, and the reaction occurred only in acid solution. But it differed from pepsin in being de stroyed by hydrochloric acid, requiring an organic acid for manifestation of its activity. Unlike trypsin it was destroyed by alkalies.

Since no proteäse resembling trypsin in its action has been demonstrated with certainty in seedlings, there is difficulty in accounting for the origin of the nitrogenous compounds detailed above, which undoubtedly arise at the cost of reserve-proteïns. Is it possible that the proteäse

reacts more energetically in the germinating seed, or must this further hydrolysis be referred to that incubus of biologists—protoplasmic activity? The question is at present open.

It has been seen that von Gorup-Besanez' statement, that the proteïnic chemolysis of germination resembles that *in vitro*, has been justified as far as its qualitative aspect is concerned. But the quantitative proportions in which the products arise during germination differ considerably from those that result from artificial decomposition. A glance at the table inserted above and its context shows that, whereas all the plants have yielded some of the characteristic products of proteohydrolysis, others have not been yet identified, although in many cases very probably present in minute amount. Also the proportion, in which those that have been identified occur, bears no resemblance to that obtaining in artificial hydrolysis. Moreover in each plant there is one predominant substance, arginin, glutamin or asparagin, the others being as a rule in quite subordinate amount. Arginin predominates in the *Coniferæ* examined, and in the footnotes to the context of the table is a list of plants in which asparagin and glutamin preponderate repectively, as also one in which such are noticed as contain both amides.

If von Gorup-Besanez' hypothesis is correct, it becomes essential that the qualitative differences and quantitative disproportions between these compounds in different species be explained. The simplest and most apparent interpretation is, that the amido-bodies arise directly in varying proportions, any one albuminoid yielding products that vary with the conditions. This view was put forward by Pfeffer some years back in a criticism of an earlier form of Schulze's present theory. The latter explained the observed phenomena through supposing that the proteohydrolysis of germination resembles both qualitatively and quantitatively that occurring *in vitro*, the products being utilised for regeneration with unlike celerity, inasmuch as the amido-acids on the whole are more adapted for this process than the amides. But since this explanation was

found in itself insufficient to account for the large accumulation of asparagin in the *Papilionaceæ*, Schulze utilised the generalisation of Borodin, that asparagin is formed constantly in metabolism, suggesting that, together with proteïnic regeneration from the amido-acids, katabolic changes occurred in the growing seedling, the amido-acids thus reformed being again consumed, and the amides neglected, so that these gradually accumulated, which accumulation has been experimentally demonstrated. Schulze criticised Pfeffer's explanation, which had at first also occurred to himself, stating that his own view was grounded on knowledge of the chemical behaviour of proteïns and the view generally accepted at that time, and not yet refuted, that the amido-compounds exist preformed in proteïns. Acceptation of this view necessitates the production of amido-substances, in so far as they are of primary origin, in the same quantitative proportions during germination as is artificial hydrolysis. Schulze also pointed out that, even if the view of the existence of preformed complexes should be given up, yet his theory would not fall since based on actual observations and not on theoretical speculations. Schulze does not deny that plants may be able to decompose proteïns in different manners, but says that this question is irrelevant, the point at issue being, whether during germination the products result in varying amount or not, and especially whether asparagin, and glutamin in *Cucurbita*, etc., can arise in such large amount as to contain a greater part of albuminoid nitrogen than all the other products together. Various reasons are given that militate against such a view, and Schulze has in the course of time materially strengthened these, so that at present the only possible conclusion is, that by far the greater part of the amides results from secondary reactions, although they are due indirectly to proteohydrolysis. He has recently shown that arginin, which is so preponderant in the *Coniferæ*, is a direct product of hydrolysis.

As a result of more extended investigations, Schulze has found it necessary to modify his theory somewhat. At an early stage he stated, that several observations pointed to the

supposition that some of the amido-compounds were converted into amides during germination, and this view has been subsequently confirmed. He holds at present that the amido-acids and bases are further decomposed with formation of nitrogenous residues, which unite with anitrogenous compounds to form the amides *synthetically*. The latter are to be regarded as the material from which the proteïns of the growing plant are produced, so that they form transitory nitrogenous reserve-substance in etiolated seedlings. This further chemolysis of the amido-compounds with formation of nitrogenous residues cannot of course be ascribed to enzymolysis; here again the physiologist must rely on the omnipotent properties of protoplasm for "explanation".

Thus asparagin can be, to a certain extent, compared with urea, as Boussingault did. Both are partly produced in proteohydrolysis, urea arising from lysatin,[1] while both are produced in major amount through synthetic processes, asparagin (resp. glutamin) from nitrogenous residues of proteohydrolysis and anitrogenous compounds, urea through union of ammonia and carbon dioxide to form ammonium carbamate, and subsequent decomposition of this.

The question of proteïnic regeneration forms still a subject of animated discussion, and no definite statement can be made as to the process. The majority of investigators, among which Boussingault, Schulze, C. O. Müller and Prianischnikow may be mentioned, deny that proteïns can be regenerated from amides in the dark, whereas Kinoshita, Hansteen and Zaleski affirm that regeneration occurs. Pfeffer and Borodin ascribe to light an indirect influence, in so far as formation of carbohydrates is concerned, while Müller asserts that its effect is direct. Also, whereas some observers, as C. O. Müller, affirm that carbohydrates are not concerned in the regeneration of proteïns, light being the determining factor, others, as Pfeffer, Borodin, Kinoshita, Hansteen and Zaleski asseverate that carbohydrates are essential to the process. E. Schulze apparently holds

[1] Lysatin is perhaps a mixture of equal molecules of arginin and lysin (Hedin); if this be so, the urea would arise from the arginin.

an intermediate position, attributing to amido-acids and amides respectively different functions in germination. He has shown that during germination the completeness and celerity of proteohydrolysis and disappearance of amido-acids is correlated with the quantity of reserve-carbohydrates, the consumption of amides being, however, dependent upon the influence of light.

Hence, as far as present knowledge extends, it may be regarded as proved, that the theory of v. Gorup-Besanez with regard to proteohydrolysis during germination is true; that amides and amido-acids functionate differently in germination, the former only resulting in small amount from direct hydrolysis, being chiefly formed synthetically from products of the further transformation of the latter and anitrogenous substances derived from the reserve-carbohydrates; that this synthesis of amides occurs in the dark: and finally that the earlier stages of proteohydrolysis, at any rate, are due to enzymic action. It is also most probable that proteïns cannot be regenerated from amides in darkness, and still nothing can be said as to the rationale of their regeneration in light.

A careful study of the principal contributions to the literature on this subject exemplifies admirably, that by systematic and continuous application most important generalisation can be effected, and phenomena elucidated that had been previously "explained" through invoking the omnipotent aid of protoplasm. An excerpt from a paper of Schulze's is of interest in this respect: "Das im Vorstehenden Mitgetheilte bitte ich zu betrachten als die Gedanken eines Chemikers, welcher sich bemüht, die chemischen Vorgänge in der Pflanze zu verstehen, ohne dass er ein 'eigenthümliches Verhalten der lebenden Eiweissmoleküle' oder ähnliche Annahmen zu Hülfe zu ziehen braucht."

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F. ESCOMBE.

SECRETION AND ABSORPTION OF GAS IN THE SWIMMING-BLADDER AND LUNGS.

PART II.—LUNGS.

IN the previous paper, I gave an account of the evidence which proves that free oxygen, and in some cases at least nitrogen, is actively secreted by the epithelium of the swimming-bladder. The present paper will deal with the much more difficult question whether the exchange of gases between the blood and the air present in the alveoli of the lungs is also brought about in whole or part by active secretion or absorption. The fact that morphologically the swimming-bladder is so closely related to the lungs increases, perhaps, the probability in favour of the existence of active secretion or absorption by the lungs. On the other hand, it must be remembered that the stream of oxygen is outwards from the blood in the swimming-bladder, while it is inwards to the blood in the lungs.

Between the air in the alveoli and the blood passing through the capillaries of the lung there is interposed practically nothing but the cells forming the capillary walls and the layer of extremely delicate flattened epithelial cells constituting the lining membrane of the alveoli. As protoplasm is semifluid in consistency, and as gases pass with the utmost readiness through water by diffusion, the most natural supposition from a purely physical standpoint is that the exchange of gases is simply due to diffusion, the epithelium playing merely a passive part in the process. Venous blood, when brought into contact with air outside the body, takes up oxygen and gives off carbonic acid, undergoing what appears to be just the same change as that brought about in blood passing through the lungs. The fact that the change occurs so much more rapidly in the lungs than in blood shaken with air outside the body is accounted for by the enormous surface presented by the alveolar walls, and the minuteness of the subdivision of the blood-stream in the lungs. The diameter of a capillary

may be taken as about ·001 mm. One cc. of blood spread out in a layer of equal thickness would cover an area of 10,000 square centimetres, or 11 square feet. Venous blood spread out in such a thin film would almost instantaneously become saturated with air. The change from venous to arterial colour can be seen to occur within a second or two even with far thicker films. It is true that in the case of such films the blood is in immediate contact with the air, while in the lungs there is a thin layer of protoplasm between the blood and the air; but the difference from a purely physical point of view is more apparent than real, since even in the case of the exposed film albuminous liquid surrounds the red corpuscles which take up nearly all the oxygen from the air.

There is thus not the slightest difficulty in imagining a physical mechanism by which the interchange of gases between the blood and the air is effected in the lung. The frequency with which formerly accepted physical theories of other physiological processes have proved on experimental investigation to be insufficient has, however, caused the diffusion theory of respiratory exchange in the lungs to be regarded with considerable suspicion; and I propose to give an outline of the results of the investigations to which these suspicions have given rise.

It is clear that if the diffusion theory be correct, the partial pressure or tension of the oxygen in solution in the blood leaving the capillaries of the lung alveoli cannot be higher than in the alveolar air: also that the tension of carbonic acid cannot be lower in the blood than in the alveolar air. Thus on the diffusion theory in arterial blood the oxygen tension ought always to be equal to or lower than, and the carbonic acid tension equal to or higher than, that of the alveolar air.

The tension of carbonic acid in blood flowing from the vessels was first investigated by Pflüger and three of his pupils[1] (Wolffberg, Strassburg, and Nussbaum), who strongly support the diffusion theory. The apparatus devised by

[1] *Pfluger's Archiv*, vol. iv., p. 465; vol. vi., pp. 23 and 65, and vol. vii., p. 296.

Pflüger for these experiments was his well-known "aerotonometer". This instrument consists essentially of a pair of glass tubes, each of which is closed by a tap above and by mercury below, and is so arranged that a stream of blood led from a vessel of the animal can be allowed to trickle slowly down the internal surface of the glass and escape under the mercury. One tube is filled before the experiment with a gas mixture containing more, and the other tube with a gas mixture containing less, carbonic acid or oxygen than corresponds to the expected tension of carbonic acid or oxygen in the blood. The tubes are kept in a water bath at the body temperature, and the pressure inside them is kept equal to that of the atmosphere. If the experiment is successful, the percentage of carbonic acid or oxygen in the first tube will diminish, and that in the second will increase, so that the percentage found afterwards will be nearly the same in both tubes. Evidently this final percentage expresses the tension of carbonic acid or oxygen in the blood in terms of the atmospheric pressure at the time of the experiment.

By means of the aerotonometer the tension of carbonic acid in the arterial blood of the dog was found by Strassburg to be from 2·1 to 3·8 per cent. (mean 2·8 per cent.) of an atmosphere. Unfortunately there is no means of determining directly the tension of carbonic acid in the alveolar air of an animal breathing normally. It is evident, however, that since the expired air consists of a mixture of alveolar air with the pure air contained in the trachea and bronchi at the end of each inspiration, the alveolar air must contain less oxygen and more carbonic acid than the expired air. If the respirations are shallow the difference between expired and alveolar air will be great, whereas with deep respirations there will be little difference. In man the expired air usually contains about 3·5 per cent. of carbonic acid, and according to the experiments of Loewy[1] the volume of the "dead space" formed by the trachea, etc., is about 140 cc., the corresponding volume of air inspired in

[1] *Pfluger's Archiv*, vol. lviii., p. 416, "Untersuchungen über die Respiration," etc., p. 26.

normal quiet inspiration being about 350 cc. The expired air thus consists of a mixture of about two parts of alveolar air, with one part of pure air; consequently it is necessary to increase by about one half the carbonic tension in expired air to obtain the alveolar carbonic acid tension. The alveolar carbonic acid will therefore be about 5 per cent. of an atmosphere in man. This tension will vary only slightly during the different phases of a respiration, since the volume of pure air introduced into the alveoli at each inspiration is only about a tenth of the total volume of air in the lungs. It is to be regretted that in the experiments from Pflüger's laboratory on the arterial carbonic acid tension in dogs no attempt was made to estimate the carbonic-acid tension of the alveolar air, or even of the expired air, during the experiments. Three analyses are, however, quoted by Wolffberg of the expired air of the dog. These gave a mean of 2·8 per cent. If we increase this value by one half to obtain the alveolar tension, as in the case of man, we get about 4 per cent. This is a good deal above the mean carbonic acid tension (2·8 per cent.) found by Strassburg in the arterial blood, and the latter value is surprisingly low on the diffusion theory. It may have been, however, that the animals on which Strassburg made his experiments, though they were not tracheotomised, were breathing so freely that the carbonic-acid tension in the alveoli was reduced to the values which he found for arterial blood.

Much more satisfactory evidence in favour of the diffusion theory was obtained in another way. The tension of carbonic acid in venous blood from the right side of the heart was measured by the aerotonometer, and found to be on an average 3·81 per cent. In the same animals, which were of course tracheotomised, the air supply to a portion of one lung was entirely cut off, and a sample of the gas withdrawn after a few minutes by means of an instrument known as the lung-catheter. On the diffusion theory the tension of carbonic acid in the gas contained in this blocked-off portion of lung ought evidently to become equal after a short interval of time to the tension of the same gases in

the venous blood. It was found by Wolffberg and Nussbaum that on an average this was the case, although in individual determinations the tension found was sometimes a good deal higher in the lung and sometimes in the blood. The average carbonic-acid tension in the blocked-off lung was 3·84 per cent., as compared with 3·81 per cent. for the venous blood.

These results evidently afford very strong support to the diffusion theory. It may be pointed out, however, that, apart from a probable source of fallacy due to the bronchial circulation, there appears to be clear evidence that the venous blood coming to the lungs was not normal venous blood, but blood in a more or less arterialised state, the difference being due to the disturbance produced by the tracheotomy and the introduction of the lung catheter. In animals which had not been tracheotomised Strassburg found that the carbonic-acid tension in the venous blood varied from about 5 to 6·5 per cent., and was thus always much higher than in the experiments just mentioned. On the hypothesis that the lung epithelium acts like that of other excretory glands, it is reasonable enough to suppose that the excretory activity would not come into play with a low carbonic-acid tension in the venous blood, or even that the epithelium might oppose the tendency of carbonic acid to escape by diffusion. The kidney epithelium, for instance, seems to regulate in the most delicate manner the percentage of water, chlorides, sugar, etc., in the blood. Thus in the case of chlorides the kidney epithelium either actively opposes their natural tendency to escape by diffusion and filtration or actively excretes them, according to their scarcity or excess in the food taken in. The normal high percentage of chlorides in the blood is thus kept very steady. A certain tension of carbonic acid in the tissues of the body may be as requisite to health as a certain tension of chlorides; hence the fact that under some conditions the evidence is distinctly against an active excretion of carbonic acid by the lungs does not exclude the hypothesis that they act towards carbonic acid like true excretory glands.

In the experiments of Pflüger and his pupils very little

attention was paid to the oxygen tension of the blood. The value of 3 to 4 per cent. obtained by Strassburg for the arterial oxygen tension was only minimal. In a later series of experiments Herter[1] obtained a much higher value, 10·4 per cent., but even this was only minimal.

The subject of the cause of gaseous exchange between the blood and the air of the lungs was next taken up by Bohr.[2] The general principle of his apparatus was the same as that of Pflüger's aerotonometer, but the blood was rendered incoagulable by a previous injection into the circulation of leech extract or "peptone," and could thus be allowed to flow continuously through the vessel containing the gas mixture and back to the animal. The object of this arrangement was to make it possible to obtain complete equilibrium between the tensions of oxygen and carbonic acid in the gas mixture and in the blood. During the experiments the amount of air per inspiration and the average composition of the expired air was determined by means of a special apparatus. The volume of the dead space formed by the contents of the tracheal cannula and trachea itself was also measured, so that it was possible to estimate the composition of the expired air at the bifurcation of the trachea. From his experiments Bohr concluded that the oxygen tension of arterial blood is frequently above, and the carbonic-acid tension frequently below, that of the expired air; and he draws the inference that the lungs are capable of actively secreting oxygen inwards and carbonic acid outwards.

Still more recently the subject was again taken up by Fredericq,[3] whose experiments were made on the same general principles, although he did not determine the composition of the expired air. His experiments showed distinctly that the oxygen tension of the blood from an artery varied from about 8 to 14 per cent. of an atmosphere, while the carbonic-acid tension varied from 1·4 to 4 per cent. The oxygen tensions found by Fredericq were thus con-

[1] *Zeitschrift für physiologische Chemie*, vol. iii., p. 98.
[2] *Skand. Archiv für Physiologie*, vol. ii., p. 236, 1891.
[3] *Archives de Biologie*, 1896, p. .

siderably lower than those usually found by Bohr, and were also lower than in expired air. His conclusion is that diffusion explains the exchange of gases between the blood and the air of the lungs, but that in the case of oxygen there is not time for the tensions in the blood and alveolar air to equalise themselves. Thus, to take an example, in experiment 5 a tension of 2·45 per cent. of carbonic acid (maximum value) was found in the arterial blood. Hence the percentage of carbonic acid in the alveolar air would be 2·45 per cent., and the corresponding alveolar-oxygen tension about 18 per cent. The oxygen tension actually found in the blood was only, however, 9·29 per cent. (maximum value). Hence equilibrium had not nearly established itself between the oxygen tensions in the blood and alveolar air. In a further series of experiments,[1] in which the animal breathed nearly pure oxygen, the difference between the tension of oxygen in the arterial blood and that calculated on the diffusion theory for the alveolar air from the carbonic-acid tension was still greater. Thus with 84·6 per. cent. of oxygen in the inspired air an oxygen tension of 56·2 per cent. and a carbonic-acid tension of 5 per cent. were found in the arterial blood. On the diffusion theory supported by Fredericq the oxygen tension of the arterial blood must thus have been 22 per cent. of an atmosphere below that of the alveolar air.

In addition to Fredericq's own experiments a further series from his laboratory were recently published by Weisberger.[2] The animal breathed into and out of a large bag. The bag and the aerotonometer were filled with the same gas mixture, and at the end the gas in the bag and in the aerotonometer was again analysed. The results were that as regards carbonic acid the gas in the aerotonometer at the end of the experiment in five cases contained from ·66 to 2·75 per cent. more carbonic acid than the air of the bag, and in six cases from 3·36 to ·18 per cent. less carbonic acid. As regards oxygen in three cases there was from 12·3 to 4·7 per cent. less oxygen in the aerotonometer, and in six

[1] *Arch. de Biologie*, 1896. [2] *Ibid.*

cases from 4·35 to ·08 per cent. more oxygen. In no case was there less carbonic acid or more oxygen in the aerotonometer at the end than at the beginning of the experiment. Weisberger considers that the results support the diffusion theory, and explains the higher percentages of oxygen and lower percentages of carbonic acid in the aerotonometer at the end of most of the experiments on the theory that the changes of composition in the air of the aerotonometer occurred so slowly that they lagged behind those of the bag. This explanation is quite a probable one, but it is evident that the results are not such as to add any very distinct support to the diffusion theory.

Fredericq's experiments were much longer than those of Bohr, so as to allow of sufficient time for equilibrium to establish itself between the gas tensions in the blood and the air of the aerotonometer. He calls special attention to the extreme slowness with which equilibrium establishes itself as regards oxygen tension, and criticises Bohr's experiments on the ground that the latter frequently did not allow sufficient time for this to occur. That such was the case in many of the experiments cannot be denied. It is nevertheless impossible to explain away Bohr's conclusions simply on this ground, as in certain experiments the oxygen in the aerotonometer air was found to have *risen* to a higher percentage than in the expired air, or the carbonic acid in the aerotonometer had *fallen* to a lower point than in the expired or tracheal air, or even, in one case, than in the inspired air. The results of the experiments in question may be quoted here.

Exp. No.	Percentage of Oxygen in				
	Inspired air.	Expired air.	Tracheal air.	Aerotonometer at beginning.	Aerotonometer at end.
1	20·9	19·18	18·11	20·40	20·44
6 (*b*)	20·9	18·43	13·52	11·64	14·35
10	20·9	19·16	15·87	17·07	17·80
13	19·99	18·78	18·55	19·20	20·67

Exp. No.	Percentage of Carbonic Acid in				
	Inspired air.	Expired air.	Tracheal air.	Aerotonometer at beginning.	Aerotonometer at end.
6	0	1·68	4·05	6·07	2·25
12	4·85	5·66	5·75	8·50	4·20

These experiments and particularly No. 12, certainly seem to afford most conclusive evidence that diffusion alone does not in all cases explain the interchange of gases in the lungs. On the other hand, it may be objected that the number of results clearly inconsistent with the diffusion theory is still too small to exclude all possibility of mistakes.

It is evident from the irregularity of the results obtained that the aerotonometer method, even in the improved form given to it by Bohr and Fredericq, presents many serious difficulties in practice, one of the chief of which is due to the fact that the animal is not under normal conditions during the experiment. Apart from these difficulties there are certain sources of fallacy which may or may not be serious, but which have not hitherto been excluded in connection with the method itself. In the first place it is by no means certain that the whole of the blood passing through the lungs is really aerated. Geppert and Zuntz,[1] whose authority on such a point cannot be questioned, bring forward very definite reasons for concluding that, during ordinary respiration at least, a small portion of the blood-stream through the lungs probably escapes aeration. If this be the case the results obtained by the aerotonometer may not be the gas tensions of the aerated blood from the lungs, but of a mixture of this blood with some venous blood. Such an admixture as Geppert and Zuntz believe to occur would not very appreciably raise the carbonic-acid tension of the blood in an artery, but might easily reduce the oxygen tension to half. From experiments by Hüfner with hæmoglobin solutions and fresh blood outside the

[1] *Pflüger's Archiv*, vol. xlii., p. 229, 1888.

body we know that the absence of as little as $\frac{1}{50}$th of the quantity of oxygen present in blood saturated with air would reduce the oxygen tension in the blood from 20·9 per cent. to half this value.

A further probable source of fallacy in the aerotonometer method lies in the fact that as proved by Pflüger a very appreciable amount of oxidation occurs spontaneously in arterial blood within an exceedingly short interval of time after it has left the lungs. The rapid darkening of the blood in consequence of this change can easily be seen in arterial blood collected over mercury, and has also been observed by Pflüger to occur in the blood within the crural artery if the latter be excluded for a few seconds. The occurrence of oxidation, to even a fraction of the extent observed by Pflüger, in the blood on its way from the lung to the aerotonometer would cause most serious fallacy.

The subject of the causes of interchange of oxygen between the blood and alveolar air was again taken up by Dr. Lorrain Smith and myself about two years ago.[1] The method we employed is a new one, and depends on the well-known fact that hæmoglobin combines with either oxygen or carbonic oxide, the carbonic-oxide compound being, however, much the more stable one. When hæmoglobin solution or undiluted blood is brought into contact with a gas mixture containing both oxygen and carbonic oxide, the hæmoglobin combines partly with the oxygen and partly with the carbonic oxide, the final result being dependent, according to a definite and sharply defined law, on the relative tensions of the two gases. Thus if the gas mixture contain 20·9 per cent. of oxygen and ·070 per cent. of carbonic oxide, the hæmoglobin shares itself equally between the two gases. The partition is the same whether, within certain limits, the atmospheric pressure or the temperature is raised or lowered, or whether by the addition or subtraction of nitrogen the percentages of oxygen and carbonic oxide are increased or diminished in corresponding

[1] *Journal of Physiology*, vol. xviii., pp. 201 and 430; vol. xx., p. 497; vol. xxii., pp. 231, 307.

proportions. An increase or diminution of the tension of one of the two gases, that of the other remaining the same, increases or diminishes in exact proportion the relative share of the hæmoglobin combining with the former gas. Thus provided we know the final state of combination of the hæmoglobin, and the percentage or tension of one of the two gases (oxygen and carbonic oxide), in a gas mixture we can infer the percentage or partial pressure of the other. The striking difference of tint between oxyhæmoglobin and carbonic-oxide hæmoglobin in dilute solution renders it easy to estimate colorimetrically the relative proportions of the two compounds present in the hæmoglobin of even a single drop of blood.

It is clear that on the diffusion theory when an animal breathes, say, ·07 per cent. of carbonic oxide in air the latter gas must continue to be absorbed by the hæmoglobin of the blood until an equilibrium establishes itself, due to the fact that the partial pressure of oxygen reached by the blood passing through the lungs is sufficient to prevent further absorption. This point will depend, firstly, on the percentage of carbonic oxide in the alveolar air, which, after absorption has ceased, will of course be the same as that of the inspired air, and, secondly, on the oxygen tension reached by the blood leaving the alveolar capillaries. If, for instance, the oxygen tension of the blood in the lungs just reaches that of the alveolar air the proportion of carbonic oxide finally absorbed by the blood will correspond, in the case of man, to what would be absorbed on shaking the blood to saturation with an atmosphere containing about 14 per cent. of oxygen and ·07 per cent. of carbonic oxide. With such an atmosphere the hæmoglobin becomes just 60 per cent. saturated with carbonic oxide. On the diffusion theory of respiratory exchange, therefore, the blood within the body ought finally to become at least 60 per cent. saturated. We have thus a means of testing the diffusion theory by a method which is free from the probable sources of fallacy connected with the aerotonometer method. Moreover, as the absorption of a moderate proportion of carbonic oxide by the blood causes

no noticeable physiological disturbance, the animal experimented on is practically under normal conditions.

Our first experiments were made on men; and we obtained the very remarkable result that with about ·07 per cent. of carbonic oxide in the air breathed, the final percentage saturation of the hæmoglobin with carbonic oxide, instead of being at least 60 per cent., was only about 33 per cent. The data of a single experiment may be given :—

Exp. No. 5. Percentage of CO = ·067 throughout.

After	1	hour	15	minutes	saturation of haemoglobin =	26·6	per cent.
„	1	„	45	„	„	29·5	„
„	2	„	11	„	„	30·5	„
„	2	„	30	„	„	30·5	„
„	3	„	10	„	„	30·5	„

Were it the case that carbonic oxide is oxidised to any appreciable extent within the body these results might still be explained consistently with the diffusion theory; for the low proportion of carbonic oxide present in the blood might be due to disappearance of carbonic oxide within the body. There is, however, evidence of the clearest kind against the occurrence of such oxidation.

A further possible hypothesis is that blood corpuscles do not behave towards oxygen and carbonic oxide in the same way inside and outside of the body. There are, however, no grounds for this supposition, and against it is the fact that the corpuscles of defibrinated blood when re-injected into the circulation appear to perform just the same functions as ordinary blood corpuscles.

It is thus very improbable that any other interpretation of our experiments is correct than that some other factor besides diffusion is concerned in the gaseous interchange between the alveolar air and the blood. Evidently, however, the results can be explained on the supposition either that the oxygen tension of the blood is raised in the lungs to beyond that of the alveolar air, or that in consequence of an active resistance offered by the epithelium the carbonic oxide tension in the blood can never rise so high as in the alveolar air. The latter supposition is very unlikely for the

following reason. We know that gases such as nitrogen or hydrogen, which are indifferent in their behaviour towards ordinary protoplasm, diffuse freely through the lung epithelium, either inwards or outwards. Carbonic oxide seems also to behave towards protoplasm as an indifferent gas. It is poisonous to vertebrate animals only because it combines with their hæmoglobin and thus cuts off the oxygen supply to their tissues; but insects, which are not dependent on hæmoglobin, live perfectly well in an atmosphere in which carbonic oxide is substituted for nitrogen; and even in the case of mammals carbonic oxide is no longer poisonous when the animal is placed in compressed oxygen, so that it obtains in simple solution in the blood sufficient oxygen to render it independent of the oxygen-carrying power of hæmoglobin.

A further reason for believing that the lung epithelium presents no obstacle to the passage inward of carbonic oxide is afforded by the fact that when air containing a small percentage of carbonic oxide is breathed nearly the whole of the gas which reaches the alveoli is absorbed, until the point is almost reached where absorption rapidly ceases in consequence of the state of equilibrium being attained.

As there can thus be hardly any doubt that carbonic oxide diffuses freely through the lung epithelium, we are driven to the conclusion that the oxygen tension is raised in the lungs to beyond that of the alveolar air. To explain the results of the above-mentioned experiments on men we must assume that the oxygen tension rose to an average of 38·5 per cent. of an atmosphere, which is about three times the alveolar oxygen tension.

A further series of experiments made on a number of different mammals and birds have fully borne out the conclusions we arrived at in the case of man. Although in different classes of animals the oxygen tensions found have differed considerably, yet in all cases they have considerably exceeded the oxygen tensions of alveolar air. The following table shows the average results for normal animals :—

Man	38·5	per cent.
Mice	23·2	„
Dogs	21·0	„
Rabbits	27·6	„
Small birds	44·6	„

We further found that by increasing or diminishing the oxygen percentage of the air a proportional increase or diminution was caused in the oxygen tension of the blood, but that when a condition was produced in which the animal began to show symptoms of suffering from want of oxygen the proportional difference between the oxygen tensions in the blood and in the alveolar air increased very markedly, indicating apparently that want of oxygen stimulates the epithelium to increased absorptive activity.

On the theory that the lung epithelium plays an active part in the absorption of oxygen it would be expected that if by any means the epithelium could be thrown out of action the laws of diffusion would then come into play alone, and the oxygen tension of the blood leaving the lungs would then fall to that of the alveolar air, or lower. During the course of our experiments we found that a fall of body temperature (which greatly diminishes the activity of all the tissues) causes the oxygen tension in the arterial blood to fall to about alveolar oxygen tension. Lorrain Smith has since found that a similar effect follows a rise of body temperature, in spite of the increased ventilation of the lungs; also that during the very acute infective process brought about in mice by inoculation with a culture of baccillus pyocyaneus a similar fall occurs. He has also succeeded in producing the same fall in arterial oxygen tension by exposing animals for a short time to oxygen at high pressure and afterwards replacing them in air. Paul Bert has shown that oxygen at high pressure is destructive to both animal and vegetable life. Hence the fact discovered by Lorrain Smith that it has a local effect on the lungs is in no way surprising. If the exposure is sufficiently long a condition of acute pneumonia is brought about, which causes the death of the animal. A short exposure is, however, sufficient to produce a condition in which the arterial oxygen tension

falls to about that of the alveolar air. The following table gives the average arterial oxygen tensions found under the conditions referred to.

Nature of abnormal condition.	Animal.	Arterial oxygen tension found.	Normal arterial oxygen tension.
Fall of body temperature .	Mice.	15·1	23·2
Rise of body temperature .	Mice.	18·2	,,
Infection with bacillus pyocyaneus . . .	Mice.	15·2	,,
Previous exposure to oxygen at high pressure .	Larks.	14·2	37
	Mice.	15·3	23·2

When instead of ordinary air a mixture rich in oxygen was breathed by mice which had previously been exposed to high-pressure oxygen, it was found that the arterial oxygen was always distinctly below that of the air breathed, and not much more than half as high as in normal animals breathing the same atmosphere.

The results of these experiments appear to exclude the possible view that for some reason or other the carbonic-oxide method gives values which are always too high in the living body. They also afford very strong support to the conclusion that the normal lung epithelium plays an active part in the taking up of oxygen. It seems clear that abnormal conditions, whether acting only locally on the lungs, or on the tissues of the body generally, very readily interfere with the normal activity of the epithelium, just as is known to occur in the case of other secretory glands.

It may further be remarked that experiments with carbonic oxide, apart from the evidence which they furnish in favour of active absorption of oxygen, strongly support the view that diffusion alone is capable of bringing about a condition of perfect equilibrium between the gas-tensions in the alveolar air and the blood passing the lungs; for even the lungs with damaged or paralysed epithelium give an oxygen tension about equal to that of alveolar air. Fredericq's conclusions that the oxygen tension of the blood leaving the lungs is considerably lower than that of alveolar air would present a good deal of difficulty even on the diffusion

theory; for if there is not time for gaseous equilibrium to establish itself between the blood and the air during rest, it is difficult to see how the blood could be completely aerated during muscular work, when the amount of oxygen absorbed by the lung in a given time is many times greater, although the alveolar oxygen-tension is about the same. Yet we know from the experiments of Geppert and Zuntz that during muscular work the blood drawn from an artery is if anything more completely aerated than during rest.

Against the probability of active absorption by the lung it might be urged that the production of a higher oxygen tension in the blood than in the alveolar air would be useless, since even at the latter oxygen tension the hæmoglobin would, practically speaking, be completely saturated with oxygen. This reasoning depends on the assumption that the only function of the oxygen absorbed by the lungs is to saturate the hæmoglobin of the venous blood. There is now, however, strong evidence to show that the oxygen has other essential functions to perform, and that at any rate the maintenance of a high oxygen tension is essential to health. We found that any great fall in the normal oxygen tension is accompanied by symptoms which cannot be attributed simply to deficient saturation of the hæmoglobin with oxygen. Possibly the high oxygen tension is in some way connected with the very considerable formation of carbonic acid which, according to the recent experiments of Bohr and Henriques, occurs within the lungs.[1]

On a review of all the investigations relating to the causes of respiratory exchange between the air in the lungs and the blood it is clear that the balance of evidence is at present strongly in favour of the view that the lung epithelium participates actively in the process. Until, however, the causes of the apparent discrepancies in the results obtained by different observers have been satisfactorily cleared up, we shall do well to regard our conclusions on this, as on so many other questions in physiology, as only provisional.

J. S. HALDANE.

[1] *Archives de Physiologie*, 1897, p. 590.

OXIDASES OR OXIDISING ENZYMES.

THE general course of the action of the enzymes or unorganised ferments is understood to be one of hydrolysis, or decomposition of the bodies attacked by them with the preliminary taking up of water into their molecule. Thus the first action of diastase on starch has been approximately expressed by the equation—

$$n C_{12}H_{20}O_{10} + H_2O = n - 1 C_{12}H_{20}O_{10} + C_{12}H_{22}O_{11};$$

that of invertase on cane sugar

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6;$$

that of emulsin on amygdalin

$$C_{20}H_{27}NO_{11} + 2H_2O = C_6H_3COH + HCN + 2(C_6H_{12}O_6).$$

Though no such equation can be written in the case of the action of pepsin on albumin or other proteids there is reason to think this also is one of hydrolysis. The most notable exception so far known is that of myrosin, which splits up sinigrine into sulphocyanate of allyl, potassic-hydrogen-sulphate and sugar, without the intervention of water.

During the last few years the researches of the French school of physiological chemists have indicated the existence of another group of enzymes, which act by promoting direct oxidation of various substances, chiefly aromatic compounds. As these enzymes appear to be distributed somewhat widely in the vegetable kingdom, the course of ferment action cannot be restricted to the single process of hydrolysis. This need not excite surprise, however, for we have long been familiar with micro-organisms which have an oxidising power. Such are the Mycoderma aceti, which forms acetic acid from alcohol, and the organisms in the soil described by Winogradsky and by Warrington, which form nitrous and nitric acids from ammonia.

LACCASE.

Of these *oxidases*, as the enzymes under discussion may be termed, the earliest one recognised was *laccase*, the body

which is concerned in the production of lacquer from the crude sap of the lac tree of South-east Asia.

The existence of this oxidase was first pointed out in 1883 by a Japanese chemist, Yoshida (1), who investigated the latex of that plant and ascertained the nature of the changes occurring in the production of the varnish.

The crude juice is obtained by making incisions into the trunks of several species of *Rhus*, and collecting the viscous matter which exudes. It has the appearance of a nearly white creamy liquid, with a faint odour resembling that of butyric acid. On exposure to air it rapidly changes colour, becoming brown and ultimately black. Spread on a flat surface it dries with a brilliant black lustre. The juice is very difficult to experiment with, as it possesses a very irritating property which affects the skin, causing painful eruptions and sores.

Yoshida states that the juice, known by the name of *urushi*, consists in great part of a peculiar acid, which he has called *urushic acid*, and to which he ascribes the formula $C_{14}H_{18}O_2$. Separated by appropriate methods from the crude latex and dried at 110° C. it forms a dark pasty substance, smelling of the original juice; it is then soluble in benzol, ether, alcohol and carbon-disulphide, but insoluble in water, and has a specific gravity of ·9851 at 23° C. When exposed to the air it does not dry or show signs of change such as the original latex does.

Besides urushic acid the crude sap contains a certain proportion of gum, and about 3·8 per cent. of a peculiar nitrogenous body, which coagulates on heating to 63° C. If the latex is treated with excess of alcohol the gum and the nitrogenous constituent are precipitated and can be removed by filtration, and the latter can be separated from the former by the action of cold water, in which it dissolves, while the gum only becomes swollen.

This nitrogenous constituent is the source of the enzyme, and on the addition of a small quantity of it to the urushic acid the latter becomes changed into the varnish. If the solution of this albuminoid matter is heated to 63° C.,

the mixture of it with urushic acid undergoes no change. The nature of this nitrogenous constituent has not been clearly established, but it seems to differ from the ordinary proteids by containing a much smaller proportion of nitrogen. Yoshida's analysis of it gives $C_{63\cdot44}H_{7\cdot41}N_{4\cdot01}O_{22\cdot94}$ Ash 1·2 in 100 parts.

From his experiments Yoshida comes to the conclusion that urushi juice consists essentially of four substances, *viz.*, urushic acid, gum, water and a peculiar enzymic matter. The phenomenon of its drying is due to the oxidation of urushic acid $C_{14}H_{18}O_2$ into oxyurushic acid $C_{14}H_{18}O_3$, which takes place by the aid of the enzyme in the presence of oxygen and moisture.

He supports this conclusion by two series of experiments which may be quoted here.

A small quantity of the original juice was put into a covered beaker, and subjected to the regulated heat of a water-bath, the water lost by evaporation being subsequently restored. The heating was carried to different temperatures and subsequently the heated juice was thinly spread over a glass plate and left to dry in a box, the air in which was kept moist. In each experiment the juice was heated for three and a half to four hours and the drying was allowed to take place at a temperature of 20° C.

The results were as follows :—

Temp. of exposure.	Subsequent time of drying.
20° C.	2 hours.
30° C.	4 ,,
40° C.	4½ ,,
55 to 59° C.	24 ,,
60 to 63° C.	Did not dry.

In the second series of experiments he found that unless moisture was present the latex did not dry; that in moist air it dried in four hours, in moist oxygen in two hours; in moist hydrogen or nitrogen it took thirty-six hours and in moist CO_2 it was dry only after two days' exposure.

It follows from these experiments that the enzyme

works energetically at a temperature of 20° C., but only when oxygen and moisture are both present; a rise of temperature above 20° C. is slowly prejudicial to it, and at 60 to 63° C. it is destroyed. It may be noted that it is at this temperature that the albuminoid matter coagulates.

Yoshida prepared oxyurushic acid from urushic acid by the action of strong chromic acid. He says that so prepared, it exhibits all the properties of the lacquer varnish.

The name *laccase* was given to the enzyme more than ten years later by Bertrand (2), who made further investigations into the peculiar behaviour of the latex and who has ascertained several additional facts about the enzyme.

In the main he confirms the earlier work of Yoshida as to the constituents of the juice. The body described as urushic acid he prefers to term *laccol*, but he has not examined it minutely on account of its deleterious properties.

He prepared the enzyme by treating the latex with a large excess of alcohol, which precipitated a gummy substance, and he purified the latter by redissolving it after filtration, and again throwing it down by the addition of ten volumes of alcohol. It separated out in white opaque flakes which yielded on hydrolysis a mixture of galactose and arabinose.

The enzyme was extracted from the gum by treatment with cold water.

In the natural juice the laccol exists in the form of an emulsion, the latter being probably due to the presence of the gum.

The laccol when separated from the latex by solution in alcohol and kept from the air, remains unchanged. If a little water is added to the solution in alcohol, a white emulsion results, which keeps for a considerable time unaltered; but if for the water a solution of laccase is substituted, the resulting emulsion turns brown at once and rapidly becomes black, especially if air is admitted.

With a boiled solution of laccase no such change can be observed.

So far as Bertrand has investigated the properties of laccol, it appears to be allied to certain polyatomic phenols. On this account he has examined the action of laccase on several of the latter, especially hydroquinone and pyrogallol (3). When the former is submitted to its influence the colour of the solution quickly becomes rose-red, and after a short interval crystalline scales with a green metallic lustre appear, the quantity rapidly increasing. When this operation is carried out in a sealed tube the oxygen present is absorbed almost completely. The liquid gives off a strong characteristic odour, and after the solid matter is separated, quinone can be extracted from it by shaking with ether. The precipitate is quinhydrone.

In the absence of the laccase, or if that employed is previously boiled for a few minutes, the hydroquinone does not absorb oxygen, nor undergo alteration. The hydroquinone is therefore oxidised by the free oxygen under the influence of the laccase.

$$2\ C_6\ H_4\ (O\ H)_2 + O_2 = 2\ H_2\ O + 2\ C_6\ H_4\ O_2$$

The colour given to the liquid is due to the formation of the quinone, and some of it, combining with the excess of hydroquinone not oxidised, produces the less soluble crystals of quinhydrone. When pyrogallol is used instead of hydroquinone, similar results are obtained, a precipitate of purpurogalline being thrown down in the form of a powder, which on heating sublimes, forming orange-red needles which are soluble in alcohol and acetic acid.

Laccase attacks many other polyphenols but only those whose phenolic oxhydriles are in the ortho- and para-positions in the benzine ring (4). Those with metaposition are affected only with difficulty. The oxidisability of these bodies by laccase seems to depend on the facility with which they can be transformed into quinones. The monophenols are not oxidised by the enzyme, but it attacks gallic acid and tannin.

Bertrand's observations on the behaviour of laccase at different temperatures do not agree with those of

Yoshida, as he finds it still active after heating it to 70° C. (5).

Bertrand has sought for laccase with some success in other plants and has indicated a rather wide distribution for it (6). In his researches he has employed the guaiacum test and appears to a certain extent to rely upon this method of recognition. This is unfortunate, as most investigators do not find it give entirely satisfactory results. He says that an alcoholic tincture of gum guaiacum turns blue in the presence of air and a little laccase; if much of the latter is present, it turns from blue to green and subsequently to yellow. In most cases however he has confirmed his results by isolating the enzyme and proving its presence by its action. This is really the only satisfactory method of proving its existence. By the two methods conjointly he has found laccase in the roots of the beet, carrot and turnip; in the tubers of the potato and the Jerusalem artichoke; in the tuberous roots of *dahlia*; in certain rhizomes; in the fruits of the apple, pear, quince and chestnut; in the vegetative parts of lucerne, clover, ryeg-rass and asparagus; and in the flowers of *Gardenia*. It may be prepared from these by extraction with water and precipitation of the extract with alcohol. If the tissue is green the extract may be saturated with chloroform and allowed to stand for twenty-four hours, after which the precipitation by alcohol may be carried out.

Rey-Pailharde has found laccase in germinating seeds, especially of plants of the Leguminosæ (7).

The activity of laccase appears to be associated in some way with the presence of manganese. Its ash always contains traces of an oxide of this metal, sometimes as much as 2 per cent. Bertrand (8) states that the activity of a preparation of the enzyme is proportional to its content of manganese.

When prepared from lucerne it is poor in this constituent, and the effect of the addition of a salt of the metal can be easily studied. Bertrand describes a typical experiment on this point. He gathered several kilograms of lucerne at the time of flowering, and bruised them in a mortar, pressing

out the sap, which was then saturated with chloroform and allowed to stand in the dark for twenty-four hours. The juice was next filtered and $2\frac{1}{2}$ volumes of alcohol added to precipitate the laccase. The precipitate was taken up with a little water, the solution filtered and the laccase again thrown down by large excess of alcohol. The final precipitate was collected and dried in vacuo. It contained a mere trace of manganese.

To 50 cc. of a solution of hydroquinone ·1 gm. of this precipitate was added, and the whole was agitated for twenty-four hours in contact with air. There was then only a red coloration produced. To a further quantity of 50 cc. of the hydroquinone solution ·1 gm. of the precipitated laccase and 1 mgr. of manganese in the form of the sulphate were added together, and in as short a time as two hours crystals of quinhydrone were formed. In the latter case there was an evident oxidation, much more extensive than after twenty-four hours' agitation in the absence of the manganese.

In an experiment so arranged that the absorption of oxygen could be measured, it was found after six hours' agitation with air at 15° C. that with laccase alone ·2 cc. oxygen were taken up; with a salt of manganese alone ·3 cc. were absorbed, but with both present together 6·3 cc. of oxygen were fixed.

The manganese is thus seen to play a very active part in the ordinary action of the enzyme. No other metal has been found to be capable of replacing it.

Manganese combined with various acid radicals was found in a further series of experiments to have a certain power of causing oxidation of hydroquinone, the protoxide appearing to act as a carrier of the oxygen (9). Comparing the action of these salts of manganese with the conjoint action of manganese and laccase, Bertrand advances the theory that the oxidases can be conceived to be special combinations of manganese with certain proteid bodies containing acid radicals, the latter varying with the particular enzyme. In such combinations the acid radical has just the necessary affinity to keep the metal in solution. The work

of conveying the oxygen would, in Bertrand's opinion, be discharged by the manganese, while the proteid matter would give to the oxidase its other characters, such as are made evident by the action of heat and the various reagents used to identify it.

Whether this hypothesis be accepted or not, it appears from the experiments that laccase at any rate is much assisted in its working by the presence of manganese, if it is not entirely dependent upon its association with that metal in some form.

Besides the plants already mentioned laccase appears to exist in a considerable number of fungi. In these plants the phenomena of oxidation are very prominent, and in consequence of this fact Bourquelot and Bertrand instituted in 1896 an investigation of them, with a view to ascertaining whether laccase or some similar enzyme plays a part in their metabolism (10). As in other cases, at the outset these observers laid considerable stress on the guaiacum reaction, and they found that the liquid that can be expressed from many fungi very rapidly oxidises the tincture with the formation of a blue colour, but that it does not bring about this change if it is first boiled. The reactions of the expressed juice with other bodies than tincture of guaiacum leave no doubt that it contains the same principle as the sap of the lacquer tree. It causes the browning of the laccol prepared from the latex of Rhus; it yields crystals of purpurogalline when allowed to act upon pyrogallol, produces quinone and quinhydrone from hydroquinone, and gives a markedly brown colour with gallic acid.

The fungus which yields laccase most readily is *Russula fœtens* Pers, one of the Basidiomycetes, which is fairly common in woods during the summer. 125 grammes of this fungus, extracted with its own weight of chloroform water yielded 60 cc. of a liquid which was at first pale yellow in colour, but which gradually reddened on exposure to air. When made to act on gallic acid in a closed flask which was constantly shaken, it was found that the oxygen was gradually absorbed, 15 cc. disappearing during the first hour of

action. It gave also the reactions just described with laccol, pyrogallol, etc.

When the extract so prepared was boiled it gradually lost its enzymic powers. Bourquelot and Bertrand say however that it is more resistant to heat than most enzymes, and that to ensure complete destruction the boiling should be maintained for a short time.

When the extract of Russula is poured into an excess of alcohol it yields only a small amount of precipitate, but this when separated off gives up the enzyme to cold distilled water. The precipitation of the laccase is not complete however when the extract is treated in this manner.

A very large number of species of fungi have been examined, chiefly belonging to the Basidiomycetes, more than half of which have been found to contain laccase, capable of acting on the aromatic bodies mentioned. Of these the genera *Russula*, *Lactarius*, *Boletus*, and *Psalliota* are the most noteworthy. The Gasteromycetes as a rule contain little if any, and the Ascomycetes and Myxomycetes, so far as they have been examined, are free or nearly free from the enzyme.

Besides working at the effect of laccase on the aromatic bodies as described above, Bourquelot and Bertrand investigated the nature of the changes of colour which supervene when many of the fleshy fungi are cut and the damaged surfaces exposed to the air (11). The tissue of Boletus almost instantaneously changes under such conditions, assuming a blue colour, the depth of tint and rapidity of appearance varying somewhat in different species. Lactarius becomes violet when wounded, while Russula turns first red and finally black.

There have been several theories as to the cause of this change of colour. Schœnbein (12) so long ago as 1856 noticed the phenomenon, and he attributed it to the action of ozone upon a particular chromogen in the fungus, saying that the latter also contains a substance capable of transforming the oxygen of the air into ozone. In 1872 Ludwig made some investigations into the subject, and con-

firmed Schœnbein as to the existence of a special chromogen in the tissue (13).

In the light of the recent work on the oxidases the authors were led to the view that one of the latter probably is concerned in the alteration of the chromogens. According to Schœnbein there is evidently something concerned besides the chromogen, and in his opinion the work effected by the particular constituent in question is the transformation of oxygen into ozone. Whatever it may be it co-operates with the oxygen of the air in causing the oxidation of the chromogen. As this is apparently the part played by laccase in the formation of the lacquer varnish, it seems probable that Schœnbein's hypothetical oxygen transformer is really an oxidising enzyme.

Working on this hypothesis Bourquelot and Bertrand carried out the following experiment. A definite weight of *Boletus cyanescens* Bull. was extracted with boiling alcohol of 95 per cent. concentration, the fungus being cut up as far as possible out of contact with air. The extraction was continued for a quarter of an hour, after which the liquid was cooled and filtered. The alcoholic extract so prepared was faintly yellow in colour, and it contained the substance which normally turns blue on exposure. It retained its colour for a considerable time, even when diluted with water and allowed to stand in contact with air.

To such an extract, diluted with its own volume of water, the investigators added a small quantity of the extract of Russula prepared as described above. In half a minute a purple coloration appeared, which passed rapidly into blue. The same effect followed on the addition of a little laccase prepared from the latex of the lacquer tree. If the enzyme was added slowly without agitation the tint was seen to be assumed gradually, the upper layers of the liquid in contact with the air being coloured first and the tint spreading thence throughout the whole.

Hence these investigators infer that the oxidase which can effect these changes is identical with the laccase of Rhus and other plants, and that in addition to acting on aromatic bodies, such as hydroquinone and pyrogallol, it

also assists to oxidise the chromogens of certain fungi, especially those which yield a blue or a red colouring matter. The laccase exists in the juice of the fungi side by side with the chromogen, but when the juice is boiled before exposure to the air has taken place the laccase is destroyed, and the chromogen in consequence remains unchanged.

An enzyme similar in many respects to laccase has been described by Piéri and Portier as existing in the gills, palpi and blood of certain molluscs (14).

TYROSINASE.

In other fungi there are different chromogens which do not turn blue on exposure to air, but become red and finally black. Of these, *Russula nigricans* Bull. is perhaps the most conspicuous example. The substance which gives rise to the black colour is almost insoluble in alcohol, but after the fungus has been boiled with this reagent it can be extracted from the residue by subsequent extraction with boiling water. When such an extract is treated with a little fresh cold water extract of the fungus, or a piece of the tissue is added to it, the liquid turns red and after a time black. If the chromogen is extracted from the fungus by boiling water and rapidly pressed, and the exuded liquor filtered and concentrated to a small bulk, it deposits colourless needle-shaped crystals, usually collected together into spheres. They are not soluble in alcohol nor readily in cold water, but they dissolve freely in hot water. They have been identified by Bertrand with *tyrosin*.

Bertrand has observed the same general course of behaviour in the expressed juice of the roots of the beet, the tuberous roots of the dahlia, and the tubers of the potato. In these cases also he has identified tyrosin in the tissues.

The similarity of behaviour to that observed in the cases of Boletus, Lactarius, etc., points to a similar cause of the change of colour. Laccase, however, has no power to set

up the blackening. Nor will simple oxidising agents bring it about. That it is an oxidation process due to the presence of a special oxidase is asserted by Bertrand, who has named the oxidase in question *tyrosinase* (15).

If to a solution of tyrosin a little of the cold water extract of Russula nigricans is added, the mixture becomes at first red, and then later assumes an inky blackness, while finally a black amorphous precipitate settles out. If this is carried out in a glass vessel without agitation, the colour first appears at the surface of the liquid. If it is conducted in a closed vessel from which air is excluded the change of colour does not take place. Nor is the change induced if the extract of the fungus is boiled before being added to the solution of tyrosin. In a closed vessel in the presence of air, the absorption of oxygen can be measured coincidently with the blackening of the liquid.

Tyrosinase can be extracted not only from Russula but from the dahlia and the beet root. It is immaterial which of the three serves as the source of the oxidase, as the effect upon the tyrosin is the same in all cases. Russula appears to contain it in greatest quantity. The same mode of extraction can be employed with either material.

In some species of Russula the two oxidases so far discussed exist side by side. Bertrand has separated them by the following treatment (5): One and a half kilogramme of freshly gathered *Russula delica* Fries. was reduced to pulp and macerated for half an hour with its own weight of chloroform water at the ordinary temperature. On pressing it, about two litres of a mucilaginous fluid were obtained, to which three litres of 95 per cent. alcohol were added. A precipitate fell which was filtered off. The liquid was concentrated to half a litre by distillation at 50° C. in vacuo, and when so obtained was found capable of acting with considerable energy on pyrogallol and hydroquinone, but to have no effect on tyrosin; it contained therefore only laccase.

The precipitate was washed with 200 cc. of chloroform water and when it was well swollen up, forming a semi-

solution, it was precipitated by addition of 400 cc. of alcohol and pressed dry. It was further purified by a repetition of this treatment. Dried at 35° C. it weighed about 7 gms. This precipitate yielded to cold water after some hours maceration, a principle which oxidised tyrosin rapidly, but had hardly any perceptible action on either hydroquinone or pyrogallol.

Tyrosinase is destroyed at a much lower temperature than laccase, being injured at about 50° C. and more rapidly at higher points; it is possible thus to prepare laccase alone from a mixture of the two, by heating the liquid containing them to 70° C. It then oxidises hydroquinone, but is without action on tyrosin.

Bourquelot (16) has recognised tyrosinase in many genera of fungi, among which may be mentioned *Boletus*, *Russula*, *Lactarius*, *Paxillus*, *Coprinus*, *Psalliota*, *Hebeloma*, *Pholiota*, *Collybia*, *Clitocybe*, *Tricholoma* and *Amanita*; in all these it is associated with laccase, but in the case of Amanita the latter enzyme is present only in small quantities.

Besides oxidising tyrosin, Bourquelot (17) has found tyrosinase to act on all the cresols, resorcinol, guaiacol, metatoluidine, xylidine, ortho-, meta-, and para-xylenol, thymol, carvacrol, and α and β naphthol. He has noted a further peculiarity in its behaviour in that it is effective when dissolved in a mixture of water and either ethyl or methyl alcohol, provided that not more than 50 per cent. of the spirit is present. The alcohols themselves are not affected by it.

ŒNOXYDASE.

Another of these oxidising enzymes has been discovered to play a prominent part in causing a particular disorder in certain wines to which the name "casse" or "cassure" has been applied. According to Bouffard (18) a wine affected in this way loses its characteristic colour, and after three or four hours it contains a red-brown precipitate. If the wine is at rest the decoloration begins at the surface where a thin pellicle of colouring matter forms, and gra-

dually the disturbance spreads to layers deeper and deeper in the liquid, until at last the walls of the vessel are covered by adherent matter, and the liquid is almost decolourised, assuming a moderately characteristic yellow tint. The deposits are formed of the colouring matter of the wine, and are insoluble in solutions of tartaric acid, even if concentrated. The changes are not attended by any evolution of gas. Bouffard says that such wines can be preserved from this disorder by heating them to 60° C. or by the addition of traces of sulphurous acid. The change is not due to bacterial action for it is not hindered by filtration through porcelain, nor by the addition of reagents which are fatal to microbes, such as salicylic acid or bichloride of mercury.

Gouirand (19) has shown that this change is due to some principle which exists in the wine itself. He took some samples of affected wine and after filtering a quantity through porcelain a large addition of alcohol threw down a precipitate of a flocculent character. When this was collected and washed, a small quantity of it added to sterilised sound wines very speedily produced the disorder. This substance was destroyed by heating. In some of Gouirand's experiments he treated samples of sound wines with a small quantity of it, and dividing them into two parts, he heated half to 80° C. In periods varying from twelve to seventy-two hours the disorder was pronounced in the unheated samples, while the controls remained clear and limpid indefinitely. Warming the controls to 60° C. gave variable results; in some it inhibited the action, in others it only slowed its progress. The substance was not affected by heating to 50° C.

When healthy wines were precipitated by alcohol in the same way as the unsound ones, the precipitate had no power of setting up the disorder when added to other samples.

Martinand has ascertained that this substance is present in ripe grapes. An extract of these gives all the reactions of laccase, oxidising hydroquinone, pyrogallol, etc., and loses the power of producing these changes if heated to

100° C. If, however, after cooling, there is added to the extract a little of the precipitate yielded when the juice of fresh grapes is treated with a large excess of alcohol, it regains the power. There is thus present in the grapes themselves, as in the wine prepared from them, a certain amount of this oxidising substance which from its behaviour must be classed with laccase and tyrosinase as an oxidising enzyme.

The name *œnoxydase* has been given to this body. It appears to resemble laccase very closely, but it is not certain that it is identical with it.

Martinand has ascertained it to be present in other fruits than grapes; plums, pears and apples especially may be mentioned. It appears to develop with the ripening of the fruit, unripe grapes containing very little. A good deal seems to be lost in the preliminary process of wine-making, wine itself containing relatively little when compared with the freshly expressed grape juice.

Martinand (21) finds that the œnoxydase can be removed from wine by shaking it with ether, which takes from it a body having some of the properties of tannin; this becomes olive-green or yellowish-brown on the addition of ferric chloride, is turned red by alkalies, and gives a white precipitate with albumin but not with gelatin. After the wine has undergone oxidation, most samples do not give up this body to ether, and many others yield only very small quantities of it.

Wine treated with ether in this way, and kept neutral, is not subject to self-oxidation.

The enzyme is possibly associated in the wine, therefore, with this body which is soluble in ether.

Martinand (20) finds that the oxidase is destroyed when its solution is heated to 72° and kept at that temperature for four minutes. Exposure to 55° C. for 1½ hours is also fatal to it. Intermediate temperatures bring about the same destruction after intermediate times of exposure.

Bouffard (22) has observed that the temperature of destruction varies a good deal under different circumstances. He has found that wines beginning to be attacked with

the disorder have been completely preserved by being heated to 60° C., and that warming them only to 55° C. materially helps them to resist it. He has further extracted normal wine by the alcohol method, and side by side with it samples of the same wine after being heated to 60° C. The precipitate in the latter case had no oxidising power, while that in the former was very active. Further investigation showed him that the nature of the medium exercised a great influence on the destruction. When the enzyme was treated in an aqueous solution of neutral reaction it withstood all temperatures below 72.5° C., but when 10 per cent. of alcohol or ·5 per cent. of tartaric acid was present destruction was complete at 52·5° C. If double these percentages of alcohol or acid were present, the necessary temperature was reduced 5° C. He agrees with Martinand, however, in saying that it can be destroyed by prolonged heating in neutral media at 60° C.

Dealing with the action of various reagents upon œnoxydase, Bouffard has ascertained that it is destroyed by the action of very dilute sulphurous acid, the necessary amount being ·02 gm. per litre of the solution of the enzyme.

Cazeneuve (23) has extracted the enzyme from unsound Beaujolais, and examined many of its properties. He precipitated the wine by excess of strong alcohol, and found the deposit was of a gummy consistency. He took up the gummy precipitate with water, and reprecipitated it with alcohol, collected the deposit rapidly, and dried it in vacuo. He found the precipitate chiefly gum, impregnated with œnoxydase.

In most respects Cazeneuve's results agree with those already quoted, but he finds further that it acts slightly on alcohols and ethers, and on the essences which give wines their peculiar bouquet (24). In its action on the wine he observes that it causes a disengagement of carbonic dioxide, and that after its action there is a diminution of the quantity of alcohol and acid. He attributes the noticeable effects produced to the action of the enzyme on the tannins. As stated above, Martinand has shown that if these are re-

moved by ether the disorder of the wine does not occur. Whether this is due to the removal of the œnoxydase with the tannin, or to the abstraction of the latter only, seems uncertain.

Cazeneuve further establishes a fact which indicates clearly that the disorder is due to the enzyme. He has submitted sound wine to the influence of a current of oxygen for some time, and also to the action of ozone, and he finds that neither process causes "la casse".

The enzyme can be preserved unchanged for some considerable time if dissolved in weak alcohol or in wine which does not contain more than 9 per cent. of spirit. It is, however, rapidly altered by strong alcohol.

It was mentioned above that Martinand had found this oxidase in the juice of apples, pears and plums. Either the same enzyme or a similar one has been described by Lindet as causing oxidation of the tannin in the cider-apple (25). If slices of apple, or a mass of the pulp, or sterilised sponges soaked in the expressed juice are placed under a bell-jar over mercury the material rapidly reddens, and there is a simultaneous absorption of oxygen and an evolution of carbonic dioxide. The phenomenon is the same if the juice in which the sterilised sponges are soaked has been filtered through porcelain, or if antiseptics are added, so that it is evident the changes are not due to the presence of micro-organisms. If boiled juice is used, it remains uncoloured, and there is no exchange of the gases mentioned. The juice may be precipitated by alcohol and the precipitate collected and washed in the usual way, and it is then found to be capable of setting up the changes in boiled juice.

It is of course a common experience that there is a marked difference in the behaviour of the pulp of the apple on exposure to air, according to whether it is raw or cooked. The latter remains uncoloured while the surface of the raw pulp soon turns a reddish-brown, particularly if it is unripe. Lindet holds that the enzyme attaches itself to the tannin, and explains the change of colour seen on wounding the fruit by the suggestion that in the intact pulp the tannin

and the enzyme are situated in different cells, being brought into contact in consequence of the wound. This suggestion seems, however, unnecessary, as the oxidases have been shown to work upon the aromatic bodies they attack only in the presence of oxygen. The access of the latter is only possible when the surface of the pulp is exposed.

ANIMAL OXIDASES.

The oxidases so far described have been discovered chiefly in the vegetable kingdom. It has been mentioned above that recently an enzyme which has similar properties to laccase is stated by Piéri and Portier (14) to exist in the gills, palpi and blood of certain molluscs. They have prepared it by methods similar to those of Bourquelot and Bertrand, and they find it oxidise various aromatic bodies in the presence of oxygen, neither the enzyme nor the gas acting in the absence of the other.

Oxidative processes have long been known to take place in blood when shed and exposed to the air. Claud Bernard first pointed out that under these conditions sugar disappeared, and his results have been confirmed by many subsequent observers. Only within recent years, however, has it been suggested that this disappearance of sugar is due to an enzymic action, but this view is now put forward by several observers.

The action was examined with some completeness by Seegen (26) in 1892, who found that the disappearance of the sugar is not influenced by the presence of chloroform, which prevents the action of living cells and micro-organisms, but does not inhibit the work of enzymes. Seegen found that the exclusion of bacteria by other means does not prevent the glycolysis, and argues in favour of the presence of a sugar-destroying enzyme. The destruction is hindered by a low temperature, occurs very rapidly at 39-40° C., and is inhibited at 54·5° C.

Lepine (27) made some experiments with the pancreas of the dog, which led him to the view that the secretion of a glycolytic enzyme is one of the functions of that

organ. He ground up the pancreas, with aseptic precautions, immediately on removal from the body, and macerated it for two to three hours at 38° C. in water containing ·2 per cent. of a mineral acid, and then neutralised the extract with sodic hydrate. To 100 cc. of the liquid resulting, he added half a gramme of glucose and digested it for an hour at 38° C. In a series of such experiments he found that there was a disappearance of sugar ranging from 10 to 50 per cent. A fresh pancreas similarly extracted with water instead of dilute acid, yielded an extract with very little power of causing destruction of sugar. Lepine inferred that from the tissue he used a glycolytic enzyme could be prepared just as similar treatment yields trypsin from the same gland.

He supported his hypothesis by an experiment, in which he compared the glycolytic power of the blood leaving the pancreas during active secretion with that possessed by it when the gland was at rest. He found that during the secretion caused by stimulation of the vagus, blood drawn from the pancreatic vein possesses little glycolytic power, but that the latter becomes considerable in the blood from the same vein during the hours immediately following the cessation of the secretion.

The power of oxidation possessed by blood was examined in 1894 by Abelous and Biarnés, who experimented on its action on salicyl-aldehyde. This body is not oxidised to salicylic acid by the air, nor by distilled water, nor by normal saline solution (a solution of sodium chloride, containing ·6 per cent. of the salt). But when defibrinated blood or blood-serum is added to the aldehyde and the mixture kept at a temperature of 37° C. the acid is formed. The oxidation was found to vary in amount with the blood of different animals.

These investigators attribute the action, as did Lepine, to the presence of a specific enzyme, which they say is destroyed by boiling.

They found that besides blood, various tissues of the body possess the same power, notably the testes, the thyroid glands, the liver, kidneys, lungs and spleen. Only when living however can they effect the destruction.

Spitzer (29) also carried out an investigation into the same subject in 1895. He says the glycolytic power is possessed not only by normal blood but that which has been treated with oxalate of potassium, and so rendered uncoagulable. Defibrinated and laky blood also have the same property. Spitzer holds that this glycolysis is not a vital process, but that the blood corpuscles excrete into the serum something which possesses the power of causing it. His work confirms that of Abelous in that he concludes that all living cells possess the property, and that it is one depending on the access of free oxygen.

Spitzer differs from the other workers quoted in not attributing the action to an enzyme, but to the activity of intramolecular oxygen, comparing it with the oxidation produced by hydrogen peroxide and other oxidising agents.

Seegen draws the opposite conclusions, and holds that the enzyme is formed by *post-mortem* changes in the blood. Arthus (30), and Lepine and Barral (31) also have advocated the view that the sugar is destroyed by an enzyme, which they hold to be formed in the white corpuscles. Arthus says that it is destroyed by warming to 55°c., and that it is not present in living blood.

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